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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C12N 15/82, 15/53, A01H 5/00		A1	(11) International Publication Number: WO 98/18948
			(43) International Publication Date: 7 May 1998 (07.05.98)
(21) International Application Number: PCT/CA97/00812		(74) Agents: GALE, Edwin, J. et al.; Kirby, Eades, Gale, Baker, Box 3432, Station D, Ottawa, Ontario K1P 6N9 (CA).	
(22) International Filing Date: 30 October 1997 (30.10.97)			
(30) Priority Data: 60/029,416 31 October 1996 (31.10.96) US		(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).	
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(54) Title: FLAX PROMOTERS FOR MANIPULATING GENE EXPRESSION			
<div><div><div>SEQ ID NO. 1</div><div>1000</div><div>1001</div><div>1002</div><div>1003</div><div>1004</div><div>1005</div><div>1006</div><div>1007</div><div>1008</div><div>1009</div><div>1010</div><div>1011</div><div>1012</div><div>1013</div><div>1014</div><div>1015</div><div>1016</div><div>1017</div><div>1018</div><div>1019</div><div>1020</div><div>1021</div><div>1022</div><div>1023</div><div>1024</div><div>1025</div><div>1026</div><div>1027</div><div>1028</div><div>1029</div><div>1030</div><div>1031</div><div>1032</div><div>1033</div><div>1034</div><div>1035</div><div>1036</div><div>1037</div><div>1038</div><div>1039</div><div>1040</div><div>1041</div><div>1042</div><div>1043</div><div>1044</div><div>1045</div><div>1046</div><div>1047</div><div>1048</div><div>1049</div><div>1050</div><div>1051</div><div>1052</div><div>1053</div><div>1054</div><div>1055</div><div>1056</div><div>1057</div><div>1058</div><div>1059</div><div>1060</div><div>1061</div><div>1062</div><div>1063</div><div>1064</div><div>1065</div><div>1066</div><div>1067</div><div>1068</div><div>1069</div><div>1070</div><div>1071</div><div>1072</div><div>1073</div><div>1074</div><div>1075</div><div>1076</div><div>1077</div><div>1078</div><div>1079</div><div>1080</div><div>1081</div><div>1082</div><div>1083</div><div>1084</div><div>1085</div><div>1086</div><div>1087</div><div>1088</div><div>1089</div><div>1090</div><div>1091</div><div>1092</div><div>1093</div><div>1094</div><div>1095</div><div>1096</div><div>1097</div><div>1098</div><div>1099</div><div>1100</div><div>1101</div><div>1102</div><div>1103</div><div>1104</div><div>1105</div><div>1106</div><div>1107</div><div>1108</div><div>1109</div><div>1110</div><div>1111</div><div>1112</div><div>1113</div><div>1114</div><div>1115</div><div>1116</div><div>1117</div><div>1118</div><div>1119</div><div>1120</div><div>1121</div><div>1122</div><div>1123</div><div>1124</div><div>1125</div><div>1126</div><div>1127</div><div>1128</div><div>1129</div><div>1130</div><div>1131</div><div>1132</div><div>1133</div><div>1134</div><div>1135</div><div>1136</div><div>1137</div><div>1138</div><div>1139</div><div>1140</div><div>1141</div><div>1142</div><div>1143</div><div>1144</div><div>1145</div><div>1146</div><div>1147</div><div>1148</div><div>1149</div><div>1150</div><div>1151</div><div>1152</div><div>1153</div><div>1154</div><div>1155</div><div>1156</div><div>1157</div><div>1158</div><div>1159</div><div>1160</div><div>1161</div><div>1162</div><div>1163</div><div>1164</div><div>1165</div><div>1166</div><div>1167</div><div>1168</div><div>1169</div><div>1170</div><div>1171</div><div>1172</div><div>1173</div><div>1174</div><div>1175</div><div>1176</div><div>1177</div><div>1178</div><div>1179</div><div>1180</div><div>1181</div><div>1182</div><div>1183</div><div>1184</div><div>1185</div><div>1186</div><div>1187</div><div>1188</div><div>1189</div><div>1190</div><div>1191</div><div>1192</div><div>1193</div><div>1194</div><div>1195</div><div>1196</div><div>1197</div><div>1198</div><div>1199</div><div>1200</div><div>1201</div><div>1202</div><div>1203</div><div>1204</div><div>1205</div><div>1206</div><div>1207</div><div>1208</div><div>1209</div><div>1210</div><div>1211</div><div>1212</div><div>1213</div><div>1214</div><div>1215</div><div>1216</div><div>1217</div><div>1218</div><div>1219</div><div>1220</div><div>1221</div><div>1222</div><div>1223</div><div>1224</div><div>1225</div><div>1226</div><div>1227</div><div>1228</div><div>1229</div><div>1230</div><div>1231</div><div>1232</div><div>1233</div><div>1234</div><div>1235</div><div>1236</div><div>1237</div><div>1238</div><div>1239</div><div>1240</div><div>1241</div><div>1242</div><div>1243</div><div>1244</div><div>1245</div><div>1246</div><div>1247</div><div>1248</div><div>1249</div><div>1250</div><div>1251</div><div>1252</div><div>1253</div><div>1254</div><div>1255</div><div>1256</div><div>1257</div><div>1258</div><div>1259</div><div>1260</div><div>1261</div><div>1262</div><div>1263</div><div>1264</div><div>1265</div><div>1266</div><div>1267</div><div>1268</div><div>1269</div><div>1270</div><div>1271</div><div>1272</div><div>1273</div><div>1274</div><div>1275</div><div>1276</div><div>1277</div><div>1278</div><div>1279</div><div>1280</div><div>1281</div><div>1282</div><div>1283</div><div>1284</div><div>1285</div><div>1286</div><div>1287</div><div>1288</div><div>1289</div><div>1290</div><div>1291</div><div>1292</div><div>1293</div><div>1294</div><div>1295</div><div>1296</div><div>1297</div><div>1298</div><div>1299</div><div>1300</div><div>1301</div><div>1302</div><div>1303</div><div>1304</div><div>1305</div><div>1306</div><div>1307</div><div>1308</div><div>1309</div><div>1310</div><div>1311</div><div>1312</div><div>1313</div><div>1314</div><div>1315</div><div>1316</div><div>1317</div><div>1318</div><div>1319</div><div>1320</div><div>1321</div><div>1322</div><div>1323</div><div>1324</div><div>1325</div><div>1326</div><div>1327</div><div>1328</div><div>1329</div><div>1330</div><div>1331</div><div>1332</di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FLAX PROMOTERS FOR MANIPULATING GENE EXPRESSIONTECHNICAL FIELD

This invention relates to gene promoters useful
5 for the genetic manipulation of plants.. More
particularly, the invention relates to gene promoters
isolated from flax useful, for example, for
manipulating the expression of indigenous genes or
transgenes in flax and other plants to modify
10 endogenous characteristics or to introduce new ones.

BACKGROUND ART

Flax (*Linum usitatissimum*) is the second most
important oilseed crop in Canada and an important crop
worldwide. Unfortunately, the use of flax seed oil is
15 limited by the narrow range of natural fatty acids
present in it. Therefore, there is a need to create
new cultivars with a wider range of fatty acid
composition to supplement the existing food and
confections markets (Rowland et al., 1995 - please
20 refer to the "References" section below for full
reference identification details). Also, there is a
commercial interest in using flax as a vehicle for
biofarming of pharmaceutical-related products by
molecular genetic manipulation of appropriate
25 transgenes (Moloney and van Rooijen, 1996). A need for
flax varieties tolerant to various abiotic and biotic
stresses has also been recognized (Rowland et al.,
1995). For example, herbicide-tolerant flax varieties
would be very useful in crop rotation programs. There
30 is always, of course, a need for promoters useful for
expressing foreign genes in various other plants.

Molecular genetic manipulation of flax seed
composition or other characteristics, such as stress
tolerance, can be achieved by expressing appropriate

transgenes using seed-specific or constitutive gene promoters. While a cDNA sequence corresponding to a flax gene has been reported (Singh et al., 1994), no promoter has yet been characterized from flax. There is, therefore, a need to identify and isolate one or more genes and promoters from flax to facilitate genetic manipulation of the flax plant and other plants.

DISCLOSURE OF INVENTION

10 An object of the invention is to identify and isolate one or more genes and promoter sequences from flax and to utilize such sequences in the genetic manipulation of plants.

Another object of the invention is to provide a
15 vector containing a promoter sequence from flax for introducing an indigenous gene or a transgene into flax or other plants.

Another object of the invention is to provide a method of modifying flax and other plants to change
20 characteristics thereof.

Stated in general terms, the present invention is based on the isolation, purification and characterization by the inventors of the present invention of two genes from flax and two promoters from
25 those genes. The sequences obtained are used for regulating the expression of a heterologous gene (foreign, reporter or transgene) in flax and other plant species. This can result in flax plants having different range of fatty acids than natural flax and
30 can result in the development of transgenic plants suitable for the production of specific products or having new and useful characteristics. Such plants and products are of commercial and industrial interest.

According to one aspect of the present invention, there is provided isolated and purified deoxyribonucleic acid of SEQ ID NO:1 or SEQ ID NO:2. These sequences relate to the novel flax genes isolated 5 and characterized by the inventors of the present invention.

These identified and isolated genes are useful in themselves for making antisense or sense constructs based on the derived sequences. Both types of construct 10 can be used to reduce the levels of similar mRNA during expression of the natural genes. This would result in an increase in 18:0 fatty acid in membrane or storage lipids in flax and other plant species. Sense constructs may also be used in enhancing the levels of 15 mRNA. Such enhancement will result in the increase of 16:1 or 18:1 fatty acids in membranes or storage lipids in flax and other plant species. Such plants will be of increased commercial interest and value.

Thus, according to another aspect of the 20 invention, there is provided a method of changing fatty acids of membrane and storage lipids of plants, characterized by making an antisense or sense construct based on SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 or SEQ ID NO:4, ligating the construct into a plant 25 transformation vector, using the vector to transform the genome of a plant or plant seed, and then growing the plant or plant seed and extracting membrane or storage lipids from the plants.

According to another aspect of the invention, 30 there is provided isolated and purified deoxyribonucleic acid of SEQ ID NO:3 or SEQ ID NO:4 (deposited as plasmids ATCC 98193 and 98192, respectively, see details below). These are the promoters that are useful for enhancing or enabling the

expression of genes introduced into flax or other plants.

According to another aspect of the invention, there is provided a gene expression cassette comprising
5 a sequence according to SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 or SEQ ID NO:4. The gene expression cassette is useful in itself as this part of the plasmids mentioned above can be used to construct other plasmid suitable to transform other plant species.

10 According to yet another aspect of the invention, there is provided a vector for introduction of a gene into a plant cell, the vector comprising a promotor of SEQ ID NO:3 or SEQ ID NO:4.

The invention also relates to transgenic plants
15 and plant seeds having a genome containing an introduced promoter sequence of SEQ ID NO:3 or SEQ ID NO:4 regulating the expression of an introduced gene, and a method of producing such plants and plant seeds.

The invention also relates to substantially
20 homologous DNA sequences (e.g. greater than or equal to 40% homology, more preferably greater than or equal to 70% homology) isolated and/or characterized by known methods using the sequence information of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 or SEQ ID NO:4, and to parts
25 of reduced length of promoter sequences SEQ ID NO:3 or SEQ ID NO:4 that are still able to function as promoters of gene expression. It will be appreciated by persons skilled in the art that small changes in the identities of nucleotides in a specific promoter
30 sequence may result in reduced or enhanced effectiveness of the promoters and that partial promoter sequences often work as effectively as the full length versions. The ways in which promoter sequences can be varied or shortened are well known to

persons skilled in the art, as are ways of testing the effectiveness of promoters. All such variations of the promoters are therefore claimed as part of the present invention.

5 It should be noted that the term "promoter" in this disclosure includes the core promoter elements (TATA box and initiator) and upstream regulatory elements (enhancers) (Datla et al., 1997).

As will be appreciated from the description above,
10 the promoters of the invention are beneficial in manipulating the expression of genes in flax and other crops.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows genomic DNA sequence of the SAD1 [SEQ
15 ID NO:1; identified in Fig. 1 as LUSAD1.SEQ] and SAD2
[SEQ ID NO:2; identified in Fig. 1 as LUSAD2.SEQ] genes and the corresponding SAD cDNA sequence [SEQ ID NO:5; identified in Fig. 1 as LUCDNA]. Nucleotides (nt) are represented by capital letters. Nucleotides different
20 from the cDNA sequence are shaded, including those of introns. Differences between SAD1 and SAD2 are shown in shaded lower case letters. Gaps in the sequences are presented by dashes. The start and stop codons on the cDNA sequence are boxed.

25 Fig. 2A is a partial restriction map of the SAD1 gene, and Fig. 2B shows the result of a DNA blot analysis identifying the regulatory sequences of SAD1 and SAD2.

Fig. 3 shows an outline of the scheme employed to
30 isolate the promoter regions of the two SAD genes.

Position and direction of the primers used in IPCR are indicated by arrowheads. Various abbreviations are as follows: E, exon; I, Intron; RE, 5'- regulatory elements (promoters); and UT, untranslated regions.

Fig. 4 discloses nucleotide sequences [SEQ ID NO:3 (SAD1) and SEQ ID NO:4 (SAD2)] of the 5'- regulatory regions of the two SAD genes. Homologous nt are represented by a dash (-), gaps by a dot (.), and additions by lower case letters. A putative transcriptional site is indicated by +1, and a TATA box is overlined. Key restriction sites are also shown.

Fig. 5 shows salient features of the plasmids CDC214 and pCDC220. Various abbreviations are as follows: flax promoter I, SAD1 gene promoter; flax promoter II, SAD2 gene promoter; *GUS* (*uidA*), gene for β -glucuronidase enzyme; *nos-T*, transcriptional terminator of the nopaline synthase gene; *nptII*, neomycin phospho-transferase expression cassette. The arrowheads indicate the direction of transcription. Key restriction sites are shown. Regions outside the left and right border (LB and RB) are that of a previously described binary plant transformation vector, pRD410 (Datla et al., 1992).

Fig. 6 shows the expression of a heterologous gene (*uidA*) by the two SAD gene promoters in various tissues of flax. Different tissues are abbreviated as YL+A, young leaves and apices; ML, mature leaves; S, stems; R, roots; B, buds; 1/2 OF, half open flower; Fl, Flower; and MS, seeds at about mid-development. Data presented are from one generation of two plants transformed with a tandem 35s promoter (2x35s), two generations of two plants transformed with pCDC214 (SAD1), and one generation of two plants transformed with pCDC220 (SAD2).

Fig. 7 shows the expression of a heterologous gene (*uidA*) by the two SAD gene promoters during flax seed development and in relation to fatty acid and protein biosyntheses. For GUS assays, data represent

one generation of two plants transformed with a tandem 35s promoter (2x35s), two generations of two plants transformed with pCDC214(SAD1), and two generations of a plant transformed with pCDC220 (SAD2). For fatty acids, three individual embryos of var. McGregor were analyzed. For protein content, data are from two transgenic plants transformed with pCDC214 and 220.

Fig. 8 shows the expression of a heterologous gene(*uidA*) by the two SAD gene promoters in tobacco leaves and mid-developmental seeds. Data represent 5 to 8 transgenic plants transformed with pCDC214 (SAD1), pCDC220 (SAD2), pRD410 (35s), and pRD420 (*uidA* alone).

Fig. 9 shows the expression of a heterologous gene(*uidA*) by the two SAD gene promoters during tobacco seed development. Various developmental stages of tobacco seeds were identified according to de Silva et al. (1992) and are abbreviated as W, white; LB, light brown; B, brown; DB, dark brown; and M, mature. Data represent 5 to 8 transgenic plants transformed with pCDC214 (SAD1), pCDC220 (SAD2), pRD410 (35s), and pRD420 (*uidA* alone).

Fig. 10 shows the expression of a heterologous gene(*uidA*) by the two SAD gene promoters in canola leaves and mature seeds. Data represent 2 to 5 plants transformed with pCDC214 (SAD1), pCDC220 (SAD2), pRD410 (35s), and untransformed plants (UT).

BEST MODES FOR CARRYING OUT THE INVENTION

In flax, endogenous SAD activity can be detected from about 10 days after pollination (dap) to seed maturity, suggesting a promoter of this gene would be useful in manipulating gene expression during seed development. Moreover, SAD has been found to be the key enzyme in manipulating the levels of saturated fatty acids in rapeseed and soybean triacylglycerols

(Knutzon et al., 1992; see Töpfer et al., 1995).

During studies carried out by the inventors aimed at diversifying flax as a crop, it was discovered that there are two SAD genes in flax. The isolation,
5 purification and characterization of these genes and their promoters is disclosed below, as well as the expression capabilities of the promoters in flax and other plant species.

The promoters developed according to the present
10 invention can be used to modify an endogenous characteristic of flax or another plant species, or to add a new characteristic. An example of a modification of an endogenous characteristic of flax is, for example, the alteration of levels of different
15 types of fatty acids in the seed oils. The introduction of a new characteristic is, for example, the production of a thermoplastic polymer in plants that normally do not produce thermoplastics. While it is normally easy to detect added characteristics, it is
20 sometimes difficult to detect altered characteristics because of natural variation of characteristics in plants. The alterations can, however, be detected by comparing the average characteristics of a statistically significant number of the plants under
25 examination with a statistically significant number of genomically-unmodified plants of the same genotype, grown under identical environmental conditions at the same time. If there is an appreciable difference in the measured characteristic, then it can be said that
30 there has been an alteration of that characteristic and that the alteration is a result of the genomic-modification.

In the case of an added characteristic, again the comparison can be made with genomically-unmodified

plants of the same genotype, again grown under identical environmental conditions at the same time.

The promoters of the present invention belong to a two-member gene family encoding the enzyme $\Delta 9$ desaturase (Stearoyl-acyl carrier protein desaturase; SAD; EC 1.14.99.6). Stearoyl-acyl carrier protein desaturase is the first enzyme in the fatty acid desaturation pathway, and it catalyzes the conversion of stearoyl-ACP(18:0-ACP) to oleoyl-ACP(18:1 $\Delta 9$ -ACP).

The promoters were isolated using the inverse polymerase chain reaction (IPCR) technique. They are capable of expressing a foreign gene, e.g. *uidA* (which encodes β -glucuronidase: GUS), in various tissues with high level of expression in seeds.

In developing seeds, both promoters showed a similar temporal expression pattern for *uidA* (measured as GUS activity). The GUS activity could be detected as early as 4 dap in developing seeds and in desiccated seeds (~50 dap) of transgenic flax. In developing seeds, the ability of the promoters to effect *uidA* gene expression correlated well with both fatty acid and protein biosyntheses and the maximum activity of GUS preceded the maximal accumulation of fatty acids and proteins.

The promoters of the invention are useful in manipulating transgene expression in a variety of tissues including seeds. Some of the products which are possible using these promoters include, but are not limited to, the following: plants with enhanced herbicide, pest, pathogen, and stress resistance; plants containing oil, protein, and carbohydrate of altered composition and content; plants with reduced anti-nutritional substances; plants producing

pharmaceutical compounds such as antibodies, neuropeptides, recombinant proteins, and biodegradable thermoplastics (Bennett, 1993; Moloney and van Rooijen, 1996; Datla et al., 1997).

5 The effectiveness of the promoters of the present invention is predictable from the effectiveness of known promoters. For example, it is well established that promoters such as cauliflower mosaic virus (CaMV) are capable of expressing a wide variety of genes in a
10 wide variety of plant species. Napin promoter (from rapeseed) has been used to express a variety of genes in canola/rapeseed (Knutzon et al., 1992; Jones et al., 1995; Dahesh et al., 1996). Phaseolin gene promoter (from bean) has also been used to express several genes
15 in rapeseed (Hitz et al., 1995). The β -conglycinin promoter (from soyabean) has been used to express genes not only in soyabean but also in Petunia (Kinney, 1997; Chen et al., 1986).

Moreover, by testing the promoters in two very
20 diverse plant species, as will become apparent from the experimental detail below, the inventors have demonstrated that the promoters would function in other diverse plant species as well.

Further demonstration of this principle can be
25 obtained from Chen ZL, Schuler MA, Beachy RN. 1986; Dehesh K, Jones A, Knutzon DS, Voelker TA. 1996; Hitz WD, Mauvis CJ, Ripp KG, Reiter RJ, DeBonte L, Chen, Z. 1995; Jones A, Davies HM, Voelker TA. 1995; Kinney, AJ. 1997; and Knutzon et al., 1992.

30 It is believed that the present invention can now best be described by presenting experimental details forming a specific illustration. It should be kept in mind, however, that the present invention is not limited to these details.

EXPERIMENTAL DETAILS

Molecular Biological Techniques

Isolation of plasmid DNA, restriction digestion, modification and ligation of DNA, PCR, gel electrophoresis, and transformation and culture of *E. coli* strains were carried out according to standard procedures (Sambrook et al., 1989). Nucleotide sequencing was performed using double stranded plasmid DNA by the dideoxy chain termination method (Sanger et al., 1977) using a Taq DYEDEOXY™ terminator cycle sequencing kit (available from Applied Biosystems) and an Applied Biosystems Model 370A Sequencer (available from Applied Biosystems). The oligodeoxy-ribonucleotides used in nucleotide sequencing, and PCR techniques were synthesised using a phosphoramidate synthesis procedure in a Biosearch 8750 DNA synthesizer (New Brunswick Scientific Co.), and purified by HPLC-based protocols (Gait, 1984). IPCR was done according to Ochman et al. (1993) and Warner et al. (1993).

Plant DNA was extracted using the protocol of Dellaporta et al. (Dellaporta et al., 1983) except that RNA was removed by adding 100 µg of RNAase B (Sigma) followed by incubation at 65°C for 20 min. The DNA was extracted once with an equal volume of phenol:chloroform (1:1, v/v) and once with an equal volume of chloroform:isoamyl alcohol (24:1, v/v). Five µg of DNA was digested with the appropriate restriction enzyme, fractionated on a 0.8% agarose gel, and pressure-blotted onto Hybond-N™ nylon membranes (Amersham) using the PosiBlot™ apparatus (Stratagene) after depurination, denaturation and neutralization of the DNA (Sambrook et al., 1989). The blotting solution contained 0.02 M NaOH and 1 M NH₄-acetate. The DNA was

immobilized on the membrane by baking the membranes at 80°C for 1 h.

A radioactive probe for identifying promoters was prepared by annealing 10 ng of oligo-29A and 30A (Table 1 below) and then filling in the ends using the Klenow fragment of DNA polymerase and random primer kit solutions (GIBCO BRL).

Table 1

10 Nucleotide sequence of various
oligonucleotides (OL) used

OL-24 (-)	5'-GAA ₁₃₇₁ ATGCCATCAT- ACTCCAATCAT-3' [SEQ ID NO:6]
OL-25 (+)	5'-GAA ₁₂₀ CCTTCAACAAC- AATGGCTCTC-3' [SEQ ID NO:7]
OL-29A (+)	5'- ₁₂₀ CCTTCAACAACAATGGCTCTCAAGC- TCAACCCAGTCACCACCTT-3' [SEQ ID NO:8]
OL-30A (-)	5'- ₁₉₄ GGAGAAGTTGTTGAGGGAGCGTGTT- GAAGGGAAGGTGGTGACTGGGTTGA-3' [SEQ ID NO:9]
OL-39 (-)	5'- ₂₅₃ TTGGTGGAGGTGGAAGTCAA-3' [SEQ ID NO:10]
OL-110 (+)	5'- ₂₆₃ AGCTAAAGAAGTCACATGGAC-3' [SEQ ID NO:11]

15 NOTE: The number in subscript corresponds to the nucleotide residue in the SAD cDNA sequence (Singh et al., 1994). + and - indicate coding and non-coding strand.

The sequence of oligo-29A corresponded to nt 120-163 of SAD cDNA (reported by Singh et al., 1994). The
20 sequence of oligo-30A corresponded to nt 145-194. In this way, radioactive probe fragments spanning 75 bps in the 5' end region of SAD cDNA were obtained.

Prehybridization was done at 65°C for 3 h in 5x SSPE, 5x Denhardt's solution, 0.5% SDS, and 500 µg of

Salmon sperm DNA (Amersham). Hybridization was done at 55°C for 18 h. The membrane was washed at room temperature in 2x SSPE and 0.1% SDS for 15 and 5 min and then at 50°C in 1x SSPE and 0.1% SDS for 10 min.

5 At this point the membrane was free of background signal. Autoradiograms were obtained by exposing the membranes for variable lengths of time to Kodak X-OMAT™ AR films with intensifying screens at -70°C.

Reporter Gene Constructs

10 A 1.747 kb DNA fragment containing only the 5'-regulatory region and a part of the untranslated region of the SAD1 gene was amplified by PCR and cloned into the pCRII vector (Invitrogen Corp). The same fragment was retrieved as an *EcoRI* fragment from the pCRII
15 vector and subsequently cloned into pBluescript™ II SK (Stratagene) to gain some cloning sites. The relevant 5'- regulatory region, approximately 1.257 kb, of the SAD2 gene was PCR-amplified but using the *pfu* DNA polymerase (Stratagene), and cloned into an *EcoRV* site
20 of the pBluescript II SK vector.

The SAD1 and SAD2 gene 5' regulatory elements were cloned into pRD420 as a *SalI*-*SmaI* fragment in front of the *uidA*. The plasmid pRD420 was obtained from Dr. R.S.S. Datla, NRC Plant Biotechnology Institute, 110
25 Gymnasium Place, Saskatoon, Saskatchewan, Canada, S7N OW9 (Datla et al., 1992). The resulting constructs were labeled as pCDC214 and pCDC220. These constructs were deposited on October 3, 1996 (tested for viability on October 9, 1996, deposit receipt dated October 10,
30 1996) under the terms of the Budapest Treaty at the American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852, USA, under deposit nos. ATCC 98193 and 98192, respectively. The plasmids CDC214 and 220

were transferred directly to *Agrobacterium* strain GV3101 containing helper plasmid pMP90 (Koncz and Schell, 1986) using a freeze-thaw method of transformation (An et al., 1988).

5 Plant Transformation

Flax seeds were surface sterilized by stirring in 70% ethanol for 2 minutes, followed by three 10 minute washes in 0.5% sodium hypochlorite (freshly diluted from the commercial product), and 5 rinses in sterile distilled water. Seeds were germinated on basal medium consisting of Murashige and Skoog (MS) major and minor salts and Gamborg vitamins (Sigma 0404), 3% sucrose and 0.8% agar. The pH of the medium was adjusted to 5.8 before autoclaving. About 10 surface-sterilized seeds were placed in each 100x15 mm plate. The plates were sealed with parafilm and placed in the dark at 22°C for 5 to 7 days.

Derivatives of *Agrobacteria tumefaciens* strain GV3101/pMP90 carrying pCDC214 and pCDC220 were grown on solidified 2x YT medium (Sambrook et al. 1989) supplemented with 50 µg/ml kanamycin and 50 µg/ml gentamycin sulfate. Single colonies from 2 to 3 day-old culture plates were used to inoculate 10 ml liquid 2x YT medium containing antibiotics as above and 20 µM acetosyringone. Cultures were grown at 28°C with rotary agitation for about 24 hours. Prior to inoculation of flax tissues, the cell concentration of the suspension was adjusted to 1×10^9 cells/ml.

The following methods for obtaining transformed flax callus were modified from Mlynárová et al. (1994).

Hypocotyls of 5-7 day aseptic flax seedlings were cut into segments 3-4 mm long. To avoid dehydration, the segments were maintained in a small volume of liquid

basal medium until all the hypocotyls were cut. The hypocotyl segments were immersed in bacterial suspension (1×10^9 cells/ml) for 30 minutes with occasional swirling. The suspension was removed by aspiration and the hypocotyl segments were transferred to sterile filter paper to remove excess liquid. The segments were placed on agar-solidified (0.8%) basal medium supplemented with $4.44 \mu\text{M}$ 6-benzylaminopurine and $0.54 \mu\text{M}$ naphthaleneacetic acid (MSD4x2 medium; Basiran et al., 1987). Maltose (3%) replaced sucrose as the carbohydrate source. About 25 explants were placed in each 100x15 mm Petri dish and maintained at 22-24°C, with a 16 h photoperiod and photon density of approximately $50 \mu\text{mol}/\text{m}^2/\text{s}$. After 2 days the segments were transferred to the same medium supplemented with $100 \mu\text{g}/\text{ml}$ kanamycin for selection of transformed cells and $200 \mu\text{g}/\text{ml}$ cefotaxime to eliminate *Agrobacteria*. The explants were maintained under the same growth conditions for 3 weeks. As a control, non-inoculated segments were treated in the same way.

Green callus formed at the cut ends of most of the inoculated hypocotyl segments, whereas little or no callus appeared on non-inoculated segments and they were completely bleached after 3 weeks on the selection medium. Callus was excised and transferred to basal medium (3% maltose) supplemented with $5 \mu\text{M}$ zeatin and antibiotics as above. Shoots regenerated from some of the calli within 3-4 weeks.

When the shoots had elongated to 0.5 to 1.0 cm, they were removed from the callus and placed in capped glass tubes (100x25 mm) containing 8 ml rooting medium: 1/2 strength MS salts, 3% sucrose, $0.1 \mu\text{M}$ IAA, 0.8% agar, pH 5.8, and $30 \mu\text{g}/\text{ml}$ kanamycin for selection of

transformed shoots. The shoots were maintained under low light ($<25 \mu\text{mol}/\text{m}^2/\text{s}$) for 6-8 days by which time some of the shoots had roots about 2-3 mm long. The plantlets were transferred to pots in the growth chamber within 10-14 days, when roots had elongated to about 2 cm and the shoots were 3-5 cm tall. Transgenic plants were grown under 18 h of light ($300-500 \mu\text{mol}/\text{m}^2/\text{s}$) and day/night temperature of 20/17°C. The plants were fertilized just before flowering with a solution containing 27 g of 15N:30P:15K supplemented with 0.9 g CuSO_4 in 9 liters of water.

Transformation of canola and tobacco were performed according to Moloney et al. (1989) and Horsch et al. (1985), respectively.

15 Tissue Sampling

Various tissues and developing seeds at different stage of development were harvested and immediately frozen in liquid N_2 and stored at -80°C until analyzed.

In progeny generations, these tissues were combined from a total of 8 plants.

Fluorimetric GUS Enzyme Assay

Fluorimetric GUS assay was done essentially according to Jefferson (1987). The assays were done in a micro well titer plate and fluorescence of the reactions was measured by CytoFluor™ II multi-well fluorescence plate reader (PerSeptive Biosystems).

Determination of Fatty Acid and Protein Content in Seeds

The fatty acid content of seeds of different ages was determined by fatty acid methyl ester analysis of seed homogenates as described previously (Taylor et al., 1992).

The same protein extracts which were used for GUS

assays were used for protein estimation. Protein concentration was determined using a modified Bradford assay method (Bio-Rad protein assay) and BSA as the standard.

5 RESULTS

Isolation and characterization of the two SAD genes

The inventors of the present invention have found that three lines of evidence prove there are two SAD genes in flax, namely: the amplification of two
10 different sized DNA fragments by PCR, the results of restriction analysis of cloned PCR products, and the results of DNA blot analysis of flax genomic DNA.

The genomic sequences of the two SAD genes were amplified by PCR. Several oligonucleotide primers were
15 synthesized based on the nucleotide sequence of the published SAD cDNA sequence (Singh et al., 1994). These primers were used in all possible combinations with flax genomic DNA as the template to amplify different segments of SAD genes. The molecular size of
20 the PCR products was determined by agarose gel electrophoresis; in most reactions two products of very similar molecular size were detected, suggesting the possibility of two SAD genes in flax. Amplification with oligo-25 and 24 (Table 1) yielded a fragment of
25 about 2.6 kb. This fragment contained the whole SAD gene as determined by sequence data.

The amplified SAD gene fragments were cloned into pCRII vector (Invitrogen Corp.). The identity of the amplified gene products was confirmed by comparison of
30 their nucleotide sequences with the SAD cDNA sequence (Singh et al., 1994). Sequence analyses indicated that the SAD1 and SAD2 genes have 97.2% similarity with each other in the coding region and 96.2% and 93.7% with the published flax cDNA sequence, respectively (Fig. 1).

It is clear that the mRNA for SAD cDNA, reported by Singh et al. (1994), was transcribed from the SAD1 gene. Some general features of the flax SAD genes have been deduced from sequence analysis. As expected on the basis of the cDNA sequence, the coding region of the gene is 1191 bps. This consists of three exons interrupted by two introns of approximately 0.6 to 0.7 kb. Exon 1 consists of 123 bp, whereas exons 2 and 3 are 507 bp and 561 bp long, respectively.

10 Verification for the presence of two SAD genes in flax comes from the analyses of two independent clones, each containing the full length gene. Although the nucleotide sequences of the coding regions are almost identical, there are several base changes. One of these has altered a restriction enzyme site, NcoI, resulting in the observation that the two clones have different restriction digestion patterns. The two clones also differ significantly in their intron sequences (Fig. 1). The different intron sequences are presumably responsible for the slight difference in the molecular size of the two PCR products generated by the same primer combination.

Identification of SAD Gene Promoter Sequences in Flax Genome

25 Genomic DNA was extracted from 7-10 days old seedlings of flax var. McGregor (obtained from Dr. G. Rowland, Crop Development Centre, 51 Campus Dr., Saskatoon, Saskatchewan S7N 5A8), digested with restriction enzyme, *Bam*HI, *Bcl*II, *Bgl*III, *Nde*I or *Sst*I, gel-fractionated and blotted onto nylon membrane for probing. These restriction enzymes would cut within the flax SAD genomic sequence as indicated in Figure 2A and elsewhere in the flax genome. When the DNA blot was hybridized with the probe, DNA fragments containing

the 5'- upstream region and a part of the 5'- untranslated and coding region of the SAD gene were expected to hybridize (Figure 2A).

The result of one such experiment is shown in Figure 2B. In each lane, two different size fragments hybridized with the probe indicating the existence of two SAD genes in flax. Singh et al. (1994) have shown only one SAD gene in flax. Since both the genes might be active, the inventors decided to isolate the 5' regulatory DNA sequences of both SAD genes.

Isolation and Characterization of Promoter Elements

5'- regulatory DNA sequences of the two SAD genes were amplified using the IPCR technique.

DNA blot analysis of the flax genome indicated that the two fragments obtained from the digestion of flax DNA with the restriction enzyme SstI would contain about 1.7 and 1.2 kb of 5' flanking regions of the SAD1 and SAD2 gene, respectively (Figs. 2B, 3 and 4). These fragments are expected to contain sufficient 5'- regulatory elements required for gene expression. SstI was used to cut the flax genomic DNA, and the circularized DNA template required for IPCR was prepared. An outline of the promoter isolation scheme is shown in Fig. 3, and is believed to be self-explanatory.

Flax genomic DNA was digested with the restriction enzyme SstI and gel fractionated. DNA fragments were isolated from a region of the agarose gel where the two promoter fragments that hybridized with the SAD probe were expected (Fig. 2B and 3). These DNA fragments were ligated at a concentration favoring the circularization of single DNA molecules (Ochman et al., 1993; Warner et al., 1993). The circularized DNA was then used as a template in the IPCR with two primers

(oligo-39 and oligo 110; Table 1). The orientation of the each member of the primer set used in the IPCR is opposite to that normally used in a regular PCR (Fig. 3). Two distinct fragments of the expected sizes, 2.2 kb and 1.7 kb, were amplified using IPCR. The untranslated region and parts of the exon 1 and exon 2 constituted the additional approximately 0.5 kb (Fig.3). The two fragments could also be digested with SstI indicating the authenticity of the PCR product.

10 The two DNA fragments were cloned in the pCRII vector (Invitrogen Corp.) and sequenced. The DNA sequence of the 5'- regulatory regions of the two SAD genes was compiled and compared (Fig. 4). The two SAD promoters are quite homologous. A large deletion of 15 368 bp in the SAD2 gene promoter (corresponding to nt 759 to 391 in the SAD1 promoter) is very conspicuous. There are a few short deletions, some substitutions and minor gaps in both the promoters. Based on the sequence data, 3'- regions of these DNA fragments were 20 matched with the 5'- coding regions of the two SAD genes, and thereby assigned the promoters to their respective SAD genes.

Expression of the β -glucuronidase Gene by Flax Promoters in Transgenic Plants

25 The ability of a promoter to regulate expression of a gene spatially and temporally can be demonstrated by using it to express a heterologous gene. To achieve this here, first, reporter gene constructs were made by fusing the promoter of the SAD1 or SAD2 gene with the 30 uidA gene (Fig. 5). These expression constructs were then used to transform flax, canola and tobacco, and independent transgenic plants of these species were obtained.

Different tissues were sampled and assayed for GUS activity to determine spatial or tissue-specific expression. Developing seeds were also collected at various stages of development to analyse the temporal expression pattern of the two promoters during seed development.

These promoters were capable of expressing the *uidA* gene in various tissues, with high level of expression in seeds (Fig. 6). In developing seeds, both the promoters showed similar temporal expression patterns for GUS (Fig. 7). The GUS activity could be detected as early as 4 dap in developing seeds and in desiccated seeds (approximately 50 dap) of transgenic flax with higher activities around mid-development (14 to 28 dap).

In tobacco, GUS activity in leaf was insignificant with both the promoters whereas in seeds GUS activity could be detected easily (Fig. 8). In developing tobacco seeds, GUS activity was highest at about mid-development (Fig. 9). In canola, GUS activity could be detected easily in both leaves and seeds (Fig. 10).

Utility of the Flax Promoters in Regulating Gene Expression

The utility of the flax promoters disclosed here is demonstrated by comparing their effect on *uidA* gene expression with both lipid and protein biosynthesis in developing flax seeds. In developing seeds, *uidA* expression correlated well with both fatty acid and protein biosynthesis (Fig. 7). In seeds, maximum expression of the *uidA* gene controlled by the SAD gene promoters preceded the maximum accumulation of fatty acids and proteins. Also, in tobacco the temporal pattern of *uidA* gene expression correlated well with the lipid biosynthesis (de Silva et al., 1992).

Therefore, these promoters are useful in manipulating gene expression in seeds. Since these promoters are also active in other tissues they are useful in manipulating gene expression in a variety of tissues.

5 Utility of SAD Genes

The utility of the genes can be demonstrated by carrying out the following predictive experiments (similar experiments have been reported in Knutzen et al., 1992; Topfer et al., 1995). Firstly, antisense or
10 sense constructs are made using the disclosed or other promoters. For example, these genes or their parts can be ligated into a SmaI restriction site of pCDC 214 or 220 (Fig. 5) or any other convenient cloning site of another plant transformation vector. These recombinant
15 plasmids can then be mobilized, for example, into an *Agrobacterium* strain which can then be used to transform a variety of plant species. Any changes in fatty acids of membrane and storage lipids can be evaluated by routine methods described in this
20 application.

Both type of constructs are expected to reduce the levels of similar mRNA during expression of the natural genes resulting in an increase of 18:0 fatty acid in membrane or storage lipids. Sense constructs can also
25 be used in enhancing the levels of mRNA. Such enhancement will likely result in the increase of 16:1 or 18:1 fatty acids in membranes or storage lipids of plants. Such plants will be of increased commercial interest and value.

30

It will be appreciated by persons skilled in the art that various modifications and alterations may be made to the present invention without departing from the general scope of the invention as defined by the

following claims. All such variations and modifications should be considered part of this invention.

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(ii) TITLE OF INVENTION: Flax Promoters For Manipulating Gene
Expression

(iii) NUMBER OF SEQUENCES: 11

(iv) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: Floppy disk
- (B) COMPUTER: IBM PC compatible
- (C) OPERATING SYSTEM: PC-DOS/MS-DOS
- (D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(vi) PRIOR APPLICATION DATA:

- (A) APPLICATION NUMBER: US 60/029,416
- (B) FILING DATE: 30-OCT-1996

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2701 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Linum usitatissimum*
- (B) STRAIN: McGregor

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

ACAACCATTC AATTCAAAAG TTTTCCAAT TTCAATTCC TCATCTGCCT TACCCATAAA	60
TCTCGACGGA CACCAAAAA CTCAGCCAGC TTGCCCCCAA ACAACAGCGC AGAAAAACCT	120
TCAACAACAA TGGCTCTCAA GCTCAACCCA GTCACCACCT TCCCTTCAAC ACGCTCCCTC	180
AACAATTCT CCTCCAGATC TCCTCGCACC TTTCTCATGG CTGCTTCCAC TTCAATTCC	240
ACCTCCACCA AGTAAGCATC TCCTCCTCCT CGGAATCTCC GCCGATTCT TTTAAGCGAT	300
TGATCGTAGA TAAATTGTC GGTGCTTAC CGTTCATCAA AATCTGCACG GTTCGTTTCT	360
TCTTCTGCGC CTAGATTGCA TTATGTCATT GTTCGCTTTC CGATTGACT GACCGACATA	420
AATCAATTCC TTTGTGTTTC ACGATTCTGG GTTTGCGCT GTAATTGATT GTCAGTGTTC	480

GCACAGGTTT CCCCTTCTCC TCCTCCGTCC ATCAAATGCA TGTTATTACC ATTTCAATTT 540
CAGTTTCCTT CTCTGAAATA TCCGTCTCTG GGAAAATAAG TCTCTGATC TACTATCCTA 600
TCAGCTTGTT TAGGAGAGGT TCGATATTCG TTTACATAAA CCAATTGGCT TACAGTCCTT 660
GAACGTCTA AATGTTGGTC GCGGTGATAA TAGGTTCTCA AAAGAGGTTT GTCTATGTTG 720
TTTGGCAAAA TCTGTTTCT GTGAATCATG TTTAAGGTCC TTGGAAGAAT GACTAATGAG 780
CTATGACATG ATTACGACGT AGTAGTTATT GAACTGCTGA TAATTCATA TAGGGGTAAC 840
TTGTTGATT GTTGGTCTAC AGGGAGGCTG AGAAGCTAAA GAAGTCACAT GGACCACCAA 900
AAGAGGTGCA TATGCAAGTG ACCCATTCCTA TGCCCCCACA GAAGCTGGAG ATATTTAAGT 960
CTCTGGAAGG TTGGGCTGAG GATGTTCTAT TACCGCACCT GAAGCCAGTT GAGAAATGCT 1020
GGCAGCCACA GGATTCCTG CCCGAACCTG AGTCGGATGG GTTCGAGGAG CAAGTGAAGG 1080
AGCTCAGGGC AAGGGCCAAA GAACTGCCCC ATGACTATTT TGTTGTGCTG GTTGGGGATA 1140
TGATCACCGA AGAAGCTCTG CCGACTTACC AGACAATGCT CAACACCCCTT GACGGGGTGA 1200
GGGACGAGAC TGGAGCCAGC CTTACGCCGT GGGCAATCTG GACAAGGGCG TGGACCGCTG 1260
AAGAGAATAG GCACGGTGAC CTTCTCAACA AGTATCTATA CCTCTCTGGA AGGGTGGACA 1320
TGAGGCAAAAT TGAAGAGACC ATTCAATATC TCATCGGCTC TGGAAATGGA TGTAATCACA 1380
TACTTCATCC TTTTCTATTA ATCTTTGGGT GAACAAAATT CACTACACTG GTAGCAGCTG 1440
AAACTTTAGA TGATTTTTTT TACTGCCTAG CTTCTATGAA ACAAACCAC GTAAGTCAAA 1500
TAGGGTTGAC AATGAGTTCA AGTGGCAAAA TTTTCTTAT ATACCAACTT CGAACCCTT 1560
TATATGACAT ACCAACTCCT AGTTCGGTGA AAATTCCTCC GTCGAAGATA TAATACTTGG 1620
ATTGGTTAAA TGAATTGTGA AAGGATACAC GTGATGTGGT CTGGAATTAA TTTGTTTGAA 1680
TGATCAGTTG GGTTCGGGCG GACAACCTG AACTGGAACC ACCCTAAGTA AATTTCTTT 1740
CTGTCTTACA AATTGAGGT TCTCCTTGAT CACCTTAGTC CATCTTAGGT TTGCCCCGTA 1800
GTAAGATCTG CATTTAGCAG TTTGTCCTGG TATCTGATAT CACTAGTATC TTTGTTTGAT 1860
TCCCTAGCAT CTCTGAAACC ATCGGACAAG TAGGTGGTTT AGGACAAATT TGTTTCATTG 1920
CGGCATTTTT TGTGTTGATC GCCGTATCAT CTGGAAGAAG CAGACAGTTT TGCAAAGTGG 1980
CATCAAGCTC AAGAAAGCAA CGGCTAGAAG AAGTTCTACA TCTGATGCTT TCCTTTTGT 2040

TCTTTGTGTG CTTTTTGGAC TTTGTTCTTT TTTCCTGTAG GATCCAAGAT CCAAAAACAG 2100
AAAACAACCC CTACCTCGGT TTCATCTACA CCTCATTCCA AGAGAGGGCA ACGTTCATCT 2160
CCCACGGAAA CACAGCCAGA CTCGCCAAGG ACCATGGGGA CATGAAGCTG GCGCAGATCT 2220
GCGGGATCAT CGCAGCAGAC GAGAAACGGC ACGAAACCGC ATACACCAAG ATCGTCGAGA 2280
AGCTCTTCGA GATCGACCCT GACGGTACAG TGCTGGCACT GGCGGACATG ATGAGGAAGA 2340
AGATATCGAT GCCCCCCCAC TTGATGTACG ATGGAGAAGA CGACAACCTC TTCGACAATT 2400
ACTCGTCAGT CGCTCAACGC ATCGGGGTGT ATACTGCCAA GGATTATGCC GATATCCTGG 2460
AGTTCCTGGT GGGGAGGTGG AAAGTGGATG CTTTACGGG GCTTCCGGG GAAGGGAACA 2520
AAGCTCAGGA TTTTGTCTGC GGGCTTCCTG CGAGGATTCG AAAGTTGGAG GAGAGGGCTG 2580
CGGGGAGGGC AAAGCAAACG TCGAAATCTG TCCCGTTCAG CTGGATCTTC AGCAGAGAAT 2640
TGGTACTCTA ATGGAGTTTG CTTGAGAGTT AGAGTGTGGA ATGATTGGAG TATGATGGCA 2700
T 2701

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2705 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Linum usitatissimum*
- (B) STRAIN: McGregor

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

ACAACCATTC AATCAATAT CTCACATTCA AGTTTTTCCA ACTTCATTT CCTCATCTGC 60
CTTACCCATA AATCTCGACA CCAAAACACT CAGCCAGCTT CGTCCCAAAC AACGCAGAAA 120

AACCTTCAAC AACAAATGGCT CTCAAGCTCA ACCCAGTCAC CACCTTCCCT TCGACCCGCT 180

CCCTCAACAA CTTCTCCTCC AGATCTCCTC GCACCTTTCT CATGGCTGCT TCCACTTTCA 240

ATTCCACTTC CACCAAGTAA GTTCCCGTCA CCATCTCCTC TTCCTCGGAA TCTCCGCCGT 300

TTCATTTAAG CGATTGATCG TAGAAAATCT GTCGGTTGCT TAGCGTTCAT TCAAATCTGC 360

GCGGTTGCTT TCTTTTCTT TCTTCAGACT GCATCATCTG CATTATGTTA TTGTTGCTTT 420

CCGATTGAC TAACCTACAT AATCAATTCC TTTGTGTTTC ACGAGTCTGG ATTTTGCGCT 480

GTAATTGATT GTCAGTGTTC GGACAGGTTT CCATTCTCTC ACCTCCGTCC ATCAAATGCA 540

TGTTATTACC TACCAATTTT AGCGTCTTTC TCTGGAAATT TCTGTCTCTG TATCTACTAT 600

CCTATTAGCT TGTTTGAGAG AGGTTCAATA TTGGTTTGCA TGAACCAAGT GGCTTACAAT 660

CCTTCAACGT TCTAAATGTT GGTCGCAGTA ACAATAGGTT CTCAAAAGAG GTTTTCTAT 720

GTTGTTTGGC AAAATCTTGT TTCTGTGAAT CATGTTAAGG TCCTGGGAAG AATGATTAAT 780

GAGCTATGAC ATGATTAAGG CGTAGTAGTT ATTGAACTGC TGATAATTCA ATATAGGGGT 840

AACTTTGTTG GTTGTGTTGT GACAGGGAGG CTGAGAAGCT AAAGAAGTCA CATGGACCAC 900

CAAAAGAGGT GCATATGCAA GTGACCCATT CCATGCCCCC ACAGAAGCTG GAGATCTTTA 960

AGTCCCTTGA AGGTTGGGCA GAGGACGTTT TGTTGCCGCA CCTGAAGCCG GTTGAGAAAT 1020

GCTGGCAGCC ACAAGATTTT CTGCCCCAAC CCGAGTCGGA TGGGTTGAG GAGCAAGTGA 1080

AGGAGCTCAG GGCAAGGGCT AAAGAACTCC CCGATGACTA TTTTGTGTG CTGGTTGGGG 1140

ATATGATCAC CGAAGAAGCT CTACCGACTT ACCAGACAAT GCTCAACACC CTTGACGGGG 1200

TGAGGGACGA GACTGGAGCC AGCCTTACGC CGTGGGCAAT CTGGACAAGG GCGTGGACCG 1260

CTGAAGAGAA TAGGCACGGT GACCTTCTCA ACAAGTATCT TTACCTCTCT GGAAGGGTGG 1320

ACATGAGGCA AATTGAAAAG ACCATTCACT ATCTCATCGG CTCTGGAATG GTATATACTC 1380

ACATCCTATC TGCCCCTTTA TCCTTTTCCA TTAATCTTTG ATTGAACAAA ATTCAATAAA 1440

CTGGTAGCTG AAACCTTAGA TGATTGTTA CTGCCTAGCT TCTATGAGAA AACCCTGAA 1500

GTCAAATAGG TTTGACAATG GGTTTAATG GAAAAAGTTT CATATACCAT CTTCCATCTA 1560

TTTTATATGA CATACCAACT TCTACTTTGG AGAAAATTCG CCGTGGATAA TCATATTATT 1620

GAAGATATAG TACTTAGTAG ATTGGTTAGA TGAAGTGTAA AACAATACAT GTGATGTCGT 1680

GTGCAATTAA TTTGTGTAAA TGATTAGCTG GGTTCGGGAC GACAAATGTG AACTGGAACC 1740
CTAGTAAACT ATGAATTGAG GTTGTCTTC ATCACTTTAT TCTGTCCTGG GTCTGTTTGC 1800
CTGTTTGCAA GATCTGCATG TAGCAGTTTG TCCTGGTATT TGCTACCAGT GGTATCTTTG 1860
TTTGATTCCC TAGCATCTCT GAAAACATCG GACCAAGTAT CTGGTTAGGA CAAATTTGGT 1920
TCATTGCGGC ATTTTTTGT TGTATCGCTG TATCGTCTGG AAGAGCAGAC AGTTTTGCAA 1980
AGTGGCATCA AGCTCAAGAA AGCAACGGCT AGAAGAAGTT CTACATCTGA TCGTTCCTT 2040
TTGTTTCTTT GTGTGCTTTT TGGACTTTGT TCTTTTGGC TGTAGGATCC AAGATCCAAA 2100
AACAGAAAAC AACCCCTACC TCGGTTTCAT CTACACCCCA TTCCAAGAGA GGGCAACGTT 2160
CATCTCCAC GGAATACGG CCAGACTCGC CAAGGACCAC GGGGACATGA AGCTGGCGCA 2220
GATCTGCGGG ATCATCGCAG CAGACGAGAA GCGGCACGAA ACAGCATACA CCAAGATCGT 2280
CGAGAAGCTC TTCGAGATCG ACCCTGACGG TACAGTGTG GCTCTGCGG ACATGATGAG 2340
GAAGAAGATA TCGATGCCCG CCCACTTGAT GTACGATGGA GAAGACGACA ACCTCTTCGA 2400
CAATTACTCG TCGGTGCTC AACGCATCGG GGTGTATACT GCCAAGGATT ATGCTGATAT 2460
CCTGGAGTTC CTGGTGGGA GGTGGAAAGT GGATGCTTTT ACGGGACTTT CCGGGGAAGG 2520
GAACAAAGCT CAGGAGTTTG TCTGTGGGCT TCCAGCGAGG ATTCGAAAAT TGGAGGAGAG 2580
GGCTGCGGGG AGGGCAAAGC AAACGTCGAA ATCTGTCCA TTCAGCTGGA TCTTCAGCAG 2640
AGAATTGGTA CTCTAATGGA GTTGCCCGA GAGTTGAGTG TGGAATGATT GGAGTATGAT 2700
GGCAT 2705

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1693 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Linum usitatissimum*
(B) STRAIN: McGregor

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

GAGCTCTCAA TGTAGTAACA CAAAGCCTTC TGTCTTCTTT CTGTAACGTT CAATGCTAGA	60
ACTTGTCTTC TTATAACTGT TTGTTTGCTT CTTACGCTAA TGTGGAGAA GGATGGAGCC	120
ACGGAGATCC CGGTAAAGCA AAGGATGGAT CGAGAGGAGA CGGTGGCTCG AGAGAACATG	180
GAAGCATTGC ACAGAGCCGT CACGTTGGAA GTGCCTCATT CGCAGGCCCC GTCTCGGTAT	240
GGAACATTG GTGGTGGTGA GGTGAAGAA GAGGAGAAAG ATGCCGTAGT TCATCATCTA	300
CTGGGATGGA TTGATCCGGC CAGCATGTTT TCCTCCCGAA ATCGACCTGT CCCTATTGAT	360
GACAATGTAA CATCAATGTC AATCTCTGCA GATATCTGTT AGGATCAGGT CATGATTCTT	420
TTTTGGTTGA TTCTTGTGAA TGTGTAACAT TGATGTAAGC TATTTGTTGT TGTAATATCT	480
GATTTTGTG TTGCTTTGAT CAATCAAATA AATCTCGTTC AACGCGATCA TAAGCCTCTT	540
TCATATTCAT TTGACGACT ATGTATAGTC GTACAAACTA TTCGGTTAAC TAATCTACAT	600
CAAGTCGGAA TTAGCTAGAC ATTGTCAAGG AGGAGGAAAA TATCAAGAAA ATTGGATGAG	660
GAAATCATAC ACCCAATTCT GAAGCTGATT CTTCATCTAT GATTTGAGT TTCGACTTTT	720
TTTGAGTCTC AACTGTGATT TCGAGTTTCG ACTTGATTG GCTCTTTGAT ATTCGAAATT	780
AAATGCCTCC AAAGTGCTCT CTACTTGCGG TTGGCCTGGT TCAATGGCGA ATCATTGAAT	840
GACAGAACTA GACAGCTACC AGGTGCAAAA AACATTTGTT AATGTCCTCT TGCATTAATG	900
TCCATGTTTT CTGCATTTTA ATCTTTCCCC AAACACCTAA TATATAGCTT CATTGATCCT	960
CCTCTCACGG TTGCAGATCT CGTTGCTGAT AACACATACA TGGCTACAAG ACTCTAAAAC	1020
GGTTCAAAGT GAAATTGTTT TGGTGGTAGA GTTGTGTGTT TGGTGACTCG AAAGTTCCTGG	1080
ATTCGAATCC AGCATTCCCC ACAAATAGA CACCAACGTA GTGTTTATTT ACCGTCTTCT	1140
ATCTTGATT GACCGAGAGT TACGATATAC TCCGACAAAA AAAGACATCT TCCACATCAT	1200
CAAATGGATC CGTAGTTAGT GCAGTGGCTC GATTAACATA AATGAAAAAA GGAAAAAATT	1260
TGCCTGAAAT CGATGCTCAA AACAAAGTAGA AATTCATTCA AACATATTTA GACAAACACG	1320

ATCATTTAGC ATCATCAAAT TAATAACAAG AGCAAACAAT AAAGCACATA GCAAAACATA 1380
CAATAGTCGT CTTGCAATGT CATATGATAA TAAGCCAGTG AAACCATGAA GCCCAAGTGA 1440
AGTGGTCAAG TGGGAGCTGA AAGCTTCCGA ACCCAAGCCC CCGCTACCGG GTTAGGACAT 1500
ACGACACGCG ACATGCTACG AAACCTAAAA ATCGGTCACG CAGTTAATGG AACAAATGAA 1560
ACGCAACGAC TATTAAGTGA CCATTTTGCA GAAATGATAT GAAAAAGTGA CCATTTAGAC 1620
AAATGAGCAA AGAAAATACA AGTGGCGAGT GCTGACATAA TAAACCGAAT GCAGGCGTTA 1680
CCATCCAATT TTA 1693

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1191 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Linum usitatissimum*
- (B) STRAIN: McGregor

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

GAGCTCTCAA TGTAGTAACA CAAACTCTTT TTTTCCATA ACGTTGAATG TTAGAACTTT 60
GTCCTTTTAT AACTGTTTCT TTCATGAAGC TGATCAGCTG ATGTTGGAGA AGGATGGAGC 120
CACGGAGATT CCTGAAAAGC AAAGGATGGA ACGAGAGGAG ACGGTGACTC GAGAGTACAG 180
GGAAGCATTG CACAGAGCTG TCACGCTTGC AGTGCCTCAT TCAGAGTTCT TGTCTCGGTA 240
TGGAACATTT AGTGGCGGTG ACGTTGAAGA AGAGGAAGAA AGATGCTATG GTTCATCATC 300
TAGTGGGAAG GATTGATCCA GCCGGCATGT TCTCCTCCCG AAATCGGGCC GTCCCAATTG 360
ATGACAATGT AACATCAATG TCAATCTCTG CAGATTTTTG TTAGCAGCAG GTCATGATTC 420

TTTTTGGTT GATTCTTG TG AATGTAAGCT ATTGTTGTT GTAATATATG CATTGATTGT 480
GATTTGTTT TAGCTTTGAT CAATGAAATA AATCTCGTTC AACCCAACCA TCAGGCTCTT 540
TCATATTCAT TTTGACGACT ATATATACAT AATCGTACAA ACTATTCGGT TAACTAATCT 600
ACAGAAAGTC GGAGTTAGCT AGAGATTGTC AAGGAGGAGG AGATCATACA CCTAATTTTG 660
AAGCTGATTC TTCATCTATG ATTTGAGTTC TTGACTTGAT TTGGCTCTTC GATATTCGAA 720
ATTAAATGCC TCAATGCCTC CAAAGTGCTC TCTACTTGCG GGTGGACCTA CAAACTAGG 780
CAAACAGGTG CAAAAACAT GTGTTTACAC GTCCATGTTA TCTTGCATTG GCCCATGTTT 840
TCTGCATTGT AAATCTTTCC CCAACACAT AGTTAGACGA AGTCGATAAT CTAGCACCAT 900
CAAATCAATA ACACGAGCAA ATAATAAAGT AAATAGTGAA ACCATGAAGC CTAATTGGTC 960
GAGTGAGCT GAAAGCTTTC ATCGGTATCG AACCCAACCC CCCCTGCTAC GAACTTAAA 1020
AATGGGTTAC GCTATTACAC TCGATAGAAC TGATGAAACG CAACGATTGT TAAGTAACCA 1080
TTTTCAGAA ACGATAATTG ACAAGTGACC ATTTGGATAA ATGACCAGGG AAAATACAAG 1140
TGCGGAGTGC TGACATAATA AACCGAATGC GGGCGTTACC ATCCAATTTT A 1191

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1371 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Linum usitatissimum*

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

CGACAACCAT TCAATTCAA AGTTTTTCCA ATTTCCATTT CCTCATCTGC CTTACCCATA 60
AATCTCGACG GACACCAAAA AACTCAGCCA GCTTGGCCCC AAACAACAGC GCAGAAAAAC 120

CTTCAACAAC AATGGCTCTC AAGCTCAACC CAGTCACCAC CTTCCCTTCA ACACGCTCCC	180
TCAACAACCTT CTCCTCCAGA TCTCCTCGCA CCTTCTCAT GGCTGCTTCC ACTTTCAGTT	240
CCACCTCCAC CAAGGAGGCT GAAGCTAAAG AAGTCACATG GACCACCAAA AGAGGTGCAT	300
ATGCAAGTGA CCCATTCCAT GCCCCACAG GAAGCTGGGA GATATTTAAG TCTCTGGGAA	360
GGTTGGGGCT GAGGGATGTT CTTATTTTCG ACCTGAAGCC AGTTGAGAAA TGCTGGCAGC	420
CACAGGATTT CCTGCCCCAA CCTGAGTCGG ATGGGTTCTGA GGAGCAAGTG AAGGAGCTCA	480
GGGCAAGGGC CAAAGAACTG CCCGATGACT ATTTTGTGT GCTGGTTGGG GATATGATCA	540
CCGAAGAAGC TCTGCCGACT TACCAGACAA TGCTCAACAC CCTTGACGGG GTGAGGGACG	600
AGACTGGAGC CAGCCTTACG CCGTGGGCAA TCTGGACAAG GGCCTGGACC GCTGAAGAGA	660
ATAGGCACGG TGACCTTCTC AACAAGTATC TATACCTCTC TGAAGGGTG GACATGAGGC	720
AAATTGAAAA GACCATTCTG TATCTCATCG GCTCTGGAAT GGATCCAAA ACAGAAAACA	780
ACCCCTACCT CGGTTTCATC TACACCTCAT TCCAAGAGAG GGCAACGTTT ATCTCCCACG	840
GAAACACAGC CAGACTCGCC AAGGACCATG GGGACATGAA GCTGGCGCAG ATCTGCGGGA	900
TCATCGCAGC AGACGAGAAA CGGCACGAAA CCGCATACAC CAAGATCGTC GAGAAGCTCT	960
TCGAGATCGA CCCTGACGGT ACAGTGCTGG CACTGGCGGA CATGATGAGG AAGAAGATAT	1020
CGATGCCCGC CCACTTGATG TACGATGGAG AAGACGACAA CCTCTTCGAC AATTACTCGT	1080
CAGTCGCTCA ACGCATCGGG GATACTGCCA AGGATTATGC CGATATCCTG GAGTTCCTGG	1140
TGGGGAGGTG GAAAGTGGAT GCTTTTACGG GGCTTTCCGG GGAAGGGAAC AAAGCTCAGG	1200
ATTTTGTCTG CGGGCTTCCT GCGAGGATTC GAAAGTTGGA GGAGAGGGCT GCGGGGAGGG	1260
CAAAGCAAAC GTCGAAATCT GTCCCGTTCA GCTGGATCTT CAGCAGAGAA TTGGTACTCT	1320
AATGGAGTTT GCTTGAGAGT AGAGTGTTGA ATGATTGGAG TATGATGGCA T	1371

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Linum usitatissimum*

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

GAAATGCCAT CATACTCCAA TCAT

24

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 24 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Linum usitatissimum*

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

GAACCTTCAA CAACAATGGC TCTC

24

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 44 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Linum usitatissimum*

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

CCTTCAACAA CAATGGCTCT CAAGCTCAAC CCAGTCACCA CCTT

44

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 50 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Linum usitatissimum*

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

GGAGAAGTTG TTGAGGGAGC GTGTTGAAGG GAAGGTGGTG ACTGGGTTGA

50

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Linum usitatissimum*

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

TTGGTGGAGG TGGAAGTGAA

20

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Linum usitatissimum*

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

AGCTAAAGAA GTCACATGGA C

21

REFERENCES OF INTEREST TO THE PRESENT INVENTION

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CLAIMS:

1. Isolated and purified deoxyribonucleic acid (DNA), characterized in that said DNA includes a sequence according to SEQ ID NO:1 or SEQ ID NO:2, or a sequence that is substantially homologous thereto.
2. Isolated and purified deoxyribonucleic acid (DNA), characterized in that said DNA includes a sequence according to SEQ ID NO:3 or SEQ ID NO:4, or a sequence that is substantially homologous thereto or that is a part of SEQ ID NO:3 or SEQ ID NO:4.
3. A vector for introducing at least one gene into plant cells, characterized in that said vector contains a promoter having a sequence according to SEQ ID NO:3 or SEQ ID NO:4, or a sequence that is substantially homologous thereto, or that is a part of SEQ ID NO:3 or SEQ ID NO:4.
4. A vector according to claim 3, characterized by further including at least one gene under expression control of said promoter.
5. Plasmid pCDC220 (ATCC 98192).
6. Plasmid pCDC214 (ATCC 98193).
7. An isolated and purified gene expression cassette characterized by containing a sequences selected from SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 and SEQ ID NO:4, or a sequence that is substantially homologous thereto, or that is a part of said sequence.

8. A plant characterized by having a genome containing an introduced nucleotide sequence of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 or SEQ ID NO:4, or a sequence that is substantially homologous thereto, or that is a part of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 or SEQ ID NO:4.
9. A plant seed characterized by having a genome containing an introduced nucleotide sequence of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 or SEQ ID NO:4, or a sequence that is substantially homologous thereto, or that is a part of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 or SEQ ID NO:4.
10. A plant as claimed in 8, characterized by exhibiting an alteration of an endogenous characteristic or an addition of a new characteristic compared to a genomically-unmodified plant of the same genotype.
11. A plant seed as claimed in 9, characterized by exhibiting an alteration of an endogenous characteristic or an addition of a new characteristic compared to a genomically-unmodified plant seed of the same genotype.
12. A method of producing transgenic plant by introducing a gene into a genome of said plant under control of a promoter, characterized in that said promoter is of SEQ ID NO:3 or SEQ ID NO:4, or a sequence that is substantially homologous thereto, or that is a part of SEQ ID NO:3 or SEQ ID NO:4 and that the said gene could of SEQ ID NO:1, SEQ ID NO:2, or a

sequence that is substantially homologous thereto, or that is a part of SEQ ID NO:1, SEQ ID NO:2.

13. A method according to claim 12, characterized in that said plant is flax.

14. A method of producing a transgenic plant seed by introducing a gene into a genome of a plant under control of a promoter to produce a transgenic plant, growing said plant and obtaining transgenic seeds therefrom, characterized in that said promoter is of SEQ ID NO:3 or SEQ ID NO:4, or is a sequence that is substantially homologous thereto, or that is a part of SEQ ID NO:3 or SEQ ID NO:4, and that the said gene could of SEQ ID NO:1, SEQ ID NO:2, or a sequence that is substantially homologous thereto, or that is a part of SEQ ID NO:1, SEQ ID NO:2.

15. A method of claim 14, characterized in that said plant is selected from flax, canola and tobacco.

16. A DNA sequence characterized in that the sequence is substantially homologous to at least a part of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 or SEQ ID NO:4, and in that said sequence has been isolated or characterized using sequence information from SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 or SEQ ID NO:4.

17. A method of changing fatty acids of membrane and storage lipids of plants, characterized by introducing an antisense or sense construct based on SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 or SEQ ID NO:4 into a plant transformation vector, using the vector to transform the genome of a plant or plant seed, and then

growing the plant or plant seed and extracting membrane or storage lipids from the plants or plant seeds.

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LUCDNA.SEQ CGACAACCATTC AATTCAA.....AAGTTTTTCCAATTTCATTTCCTCATCT 60
 LUSAD1.SEQ ..ACAACCATTC AATTCAA.....AAGTTTTTCCAATTTCATTTCCTCATCT
 LUSAD2.SEQ ..ACAACCATTC AATTCAATATCTC AATTCAAGTTTTTCCAACCTCCATTTCCTCATCT

LUCDNA.SEQ GCCTTACCCATAAATCTCGACGGACACCAAAAACTCAGCCAGCTTGCCCCCAAACAACA 120
 LUSAD1.SEQ GCCTTACCCATAAATCTCGACGGACACCAAAAACTCAGCCAGCTTGCCCCCAAACAACA
 LUSAD2.SEQ GCCTTACCCATAAATCTC...GACACCAAAACACTCAGCCAGCTTcgtCCCAAACAAC.

LUCDNA.SEQ GCGCAGAAAAACCTTCAACAACAATGGCTCTCAAGCTCAACCCAGTCACCACCTTCCCTT 180
 LUSAD1.SEQ GCGCAGAAAAACCTTCAACAACAATGGCTCTCAAGCTCAACCCAGTCACCACCTTCCCTT
 LUSAD2.SEQ ..GCAGAAAAACCTTCAACAACAATGGCTCTCAAGCTCAACCCAGTCACCACCTTCCCTT

LUCDNA.SEQ CAACACGCTCCCTCAACAACCTTCTCCTCCAGATCTCCTCGCACCTTTCTCATGGCTGCTT 240
 LUSAD1.SEQ CAACACGCTCCCTCAACAACCTTCTCCTCCAGATCTCCTCGCACCTTTCTCATGGCTGCTT
 LUSAD2.SEQ CgACcCGCTCCCTCAACAACCTTCTCCTCCAGATCTCCTCGCACCTTTCTCATGGCTGCTT

LUCDNA.SEQ CCACTTTCAGTTCACCTCCACCAAG..... 300
 LUSAD1.SEQ CCACTTTC AATTCCACCTCCACCAAGTAAG.....CATCTCCTCCTCCTCGGAAT
 LUSAD2.SEQ CCACTTTC AATTCCACCTCCACCAAGTAAGTTCCCGTCACCATCTCCTCTCCTCGGAAT

LUCDNA.SEQ 360
 LUSAD1.SEQ CTCGCCCGATTCTTTTAAGCGATTGATCGTAGATAAATTTGTCGGTTGCTTACCGTTCA
 LUSAD2.SEQ CTCGCCCG. TTTCAATTTAAGCGATTGATCGTAGA. AAATCTGTCGGTTGCTTAgCGTTCA

LUCDNA.SEQ 420
 LUSAD1.SEQ TCAAAATCTGCACGGTTCGTTTCTTCTGCGCCTAGATTGCAT.....TATGTC
 LUSAD2.SEQ TCAAAATCTGCgCGGTTTCGTTTCTTCTTCTTctctcAGAcTGCATCATCTGCATTATGTT

LUCDNA.SEQ 480
 LUSAD1.SEQ ATTGTTTCGCTTTCCGATTGACTGACCGACATAAATCAATTCCCTTTGTTGTTTCAGGATTC
 LUSAD2.SEQ ATTGTTTCG. TTTCCGATTGACTaACCTACATAA. TCAATTCCTTTGTTGTTTCAGGATTC

LUCDNA.SEQ 540
 LUSAD1.SEQ TGGGTTTTCGCTGTAATTGATTGTGAGTGTGTCACAGGTTCCCTTCTCCTCCTCCG
 LUSAD2.SEQ TGGGTTTTCGCTGTAATTGATTGTGAGTGTGgACAGGTTTCatTTCTCCaCCTCCG

LUCDNA.SEQ 600
 LUSAD1.SEQ TCCATCAAATGCATGTTATTACCATTTCAATTTAGTTTCCTTCTCTGAAATATCCGTCT
 LUSAD2.SEQ TCCATCAAATGCATGTTATTACC. TaccAAATTCAGcTCTTCTCTGgAA....aTtT

LUCDNA.SEQ 660
 LUSAD1.SEQ CTGGGAAATAAGTCTCTGTATCTACTATCCTATCAGCTTGTTTAGGAGAGGTTTCGATAT
 LUSAD2.SEQ CTG.....TCTCTGTATCTACTATCCTATTAGCTTGTTTgaGAGAGGTTCaATAT

LUCDNA.SEQ 720
 LUSAD1.SEQ TCGTTTACATAAACC AATTGGCTTACAGTCCTTGAACGTTCTAAATGTTGGTCGCGGTGA
 LUSAD2.SEQ TgGTTTgCATgAACCAAgTGGCTTACaATCCTTCAACGTTCTAAATGTTGGTCGCaGTaA

LUCDNA.SEQ 780
 LUSAD1.SEQ TAATAGGTTCTCAAAGAGGTTTGTCTATGTTGTTTGGCAAAATCTTGTTCGTGAATC
 LUSAD2.SEQ caATAGGTTCTCAAAGAGGTTTtTCTATGTTGTTTGGCAAAATCTTGTTCGTGAATC

LUCDNA.SEQ 840
 LUSAD1.SEQ ATGTTTAAGGTCCTTGGAAGAATGACTAATGAGCTATGACATGATTACGACGTAGTAGTT
 LUSAD2.SEQ ATGTT. AAGGTCCTgGGAAGAATGatTAATGAGCTATGACATGATTaAgCGTAGTAGTT

LUCDNA.SEQ 900
 LUSAD1.SEQ ATTGAACTGCTGATAATTCAATATAGGGGTAACCTTTGTTGATTGTTTGGTCACAGGGAGG
 LUSAD2.SEQ ATTGAACTGCTGATAATTCAATATAGGGGTAACCTTTGTTGgTTGTTTGGTgACAGGGAGG

Figure 1

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LUCDNA.SEQ CTGA..AGCTAAAGAAGTCACATGGACCACCAAAGAGGTGCATATGCAAGTGACCCATT 960
 LUSAD1.SEQ CTGAGAAGCTAAAGAAGTCACATGGACCACCAAAGAGGTGCATATGCAAGTGACCCATT
 LUSAD2.SEQ CTGAGAAGCTAAAGAAGTCACATGGACCACCAAAGAGGTGCATATGCAAGTGACCCATT

LUCDNA.SEQ CCATGCCCCCAGGAAGCTGGGAGATATTTAAGTCTCTGGGAAGGTTGGGGCTGAGGGA 1020
 LUSAD1.SEQ CCATGCCCCCAG .AAGCTGG .AGATATTTAAGTCTCTGG .AAGGTTGGG .CTGAGG .A
 LUSAD2.SEQ CCATGCCCCCAG .AAGCTGG .AGATcTTTAAGTCCCTtG .AAGGTTGGG .cagaGGA

LUCDNA.SEQ TGTTCCTATTTTCGCACCTGAAGCCAGTTGAGAAATGCTGGCAGCCACAGGATTTCTTGCC 1080
 LUSAD1.SEQ TGTTCCTATTACCGCACCTGAAGCCAGTTGAGAAATGCTGGCAGCCACAGGATTTCTTGCC
 LUSAD2.SEQ cGTTCTgTTgCCGCACCTGAAGCCgGTTGAGAAATGCTGGCAGCCACAaGATTTCTTGCC

LUCDNA.SEQ CGAACCTGAGTCGGATGGGTTTCGAGGAGCAAGTGAAGGAGCTCAGGGCAAGGGCCAAAGA 1140
 LUSAD1.SEQ CGAACCTGAGTCGGATGGGTTTCGAGGAGCAAGTGAAGGAGCTCAGGGCAAGGGCCAAAGA
 LUSAD2.SEQ CGAACcGAGTCGGATGGGTTTCGAGGAGCAAGTGAAGGAGCTCAGGGCAAGGGcAAAGA

LUCDNA.SEQ ACTGCCCGATGACTATTTTGTGTGCTGGTTGGGGATATGATCACCGAAGAAGCTCTGCC 1200
 LUSAD1.SEQ ACTGCCCGATGACTATTTTGTGTGCTGGTTGGGGATATGATCACCGAAGAAGCTCTGCC
 LUSAD2.SEQ ACTcCCCGATGACTATTTTGTGTGCTGGTTGGGGATATGATCACCGAAGAAGCTCTaCC

LUCDNA.SEQ GACTTACCAGACAATGCTCAACACCCTTGACGGGGTGAGGGACGAGACTGGAGCCAGCCT 1260
 LUSAD1.SEQ GACTTACCAGACAATGCTCAACACCCTTGACGGGGTGAGGGACGAGACTGGAGCCAGCCT
 LUSAD2.SEQ GACTTACCAGACAATGCTCAACACCCTTGACGGGGTGAGGGACGAGACTGGAGCCAGCCT

LUCDNA.SEQ TACGCCGTGGGCAATCTGGACAAGGGCGTGGACCGCTGAAGAGAATAGGCACGGTGACCT 1320
 LUSAD1.SEQ TACGCCGTGGGCAATCTGGACAAGGGCGTGGACCGCTGAAGAGAATAGGCACGGTGACCT
 LUSAD2.SEQ TACGCCGTGGGCAATCTGGACAAGGGCGTGGACCGCTGAAGAGAATAGGCACGGTGACCT

LUCDNA.SEQ TCTCAACAAGTATCTATACCTCTCTGGAAGGGTGGACATGAGGCAAATTGAAAAGACCAT 1380
 LUSAD1.SEQ TCTCAACAAGTATCTATACCTCTCTGGAAGGGTGGACATGAGGCAAATTGAAAAGACCAT
 LUSAD2.SEQ TCTCAACAAGTATCTtTACCTCTCTGGAAGGGTGGACATGAGGCAAATTGAAAAGACCAT

LUCDNA.SEQ TCAGTATCTCATCGGCTCTGGAATGG..... 1440
 LUSAD1.SEQ TCAGTATCTCATCGGCTCTGGAATGGTATGTAATCACATACT.....TCATCCTT
 LUSAD2.SEQ TCAGTATCTCATCGGCTCTGGAATGGTATaTAcTACATCCTATCTGCCCTTtATCCTT

LUCDNA.SEQ 1500
 LUCAD1.SEQ TTCTATTAAATCTTTGGGTGAACAAAATTCACTACACTGGTAGCAGCTGAAACTTTAGATG
 LUCAD2.SEQ TTCCaTTAAATCTTTGatTGAACAAAATTCAaTaaACTGGTAGC...TGAAACTTTAGATG

LUCDNA.SEQ 1560
 LUSAD1.SEQ ATTTTITTTTACTGCCTAGCTTCTATGAAACAAACCACGTAAGTCAAATAGGGTTGACAA
 LUSAD2.SEQ ATTTtGt..TACTGCCTAGCTTCTATGaga..AAACCActgAAGTCAAATAGGTTTGACAA

LUCDNA.SEQ 1620
 LUSAD1.SEQ TGAGTTCAAGTGGCAAAATTTTCTTATATACCAACTTCGAACCACTTTATATGACATAC
 LUSAD2.SEQ TGgGTTtAAaTGGaAAAAGTT...TcATATACCACTTTCaAtCtAtTTTATATGACATAC

LUCDNA.SEQ 1680
 LUSAD1.SEQ CAACTCCTAGTTCGGTTAAAATTCTCTC.....CGTCGAAGATATAATACTT
 LUSAD2.SEQ CAACTtCTAcTtTGgagAAAATTGcCGTGGATAATCATATtAtTGAAGATATAgTACTT

LUCDNA.SEQ 1740
 LUSAD1.SEQ GG...ATTGGTTAAATGAATTGTGAAAGGATACACGTGATGTGGTCTGGAATTAATTTGT
 LUSAD2.SEQ aGTAGATTGGTTAgATGAActGTTtAAAcAATACatGTGATGTcGTgTGcAATTAATTTGT

Figure 1 (cont.)

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LUCDNA.SEQ ..... 1800
LUSAD1.SEQ TTGAATGATCAGTTGGGTTTCGGGGCGACAACCTGTGAACTGGAACCCCTAAGTAAATTT
LUSAD2.SEQ gTaaATGATtAGcTGGGTTTCGGGgCGACAaTGTGAACTGGAA...CCCTA.GTAAA...

LUCDNA.SEQ ..... 1860
LUSAD1.SEQ TCTTTCTGTCTACAAATTTGAGGTTCTCCTTGATCACCTTAGTCCATCTTAGGT....T
LUSAD2.SEQ .....CTATgAATT.GAGGTTgTCCTTcATCACtTTAtTctgTccTgGGTCTGTT

LUCDNA.SEQ ..... 1920
LUSAD1.SEQ TGCCCGTTAGTAAGATCTGCATTTAGCAGTTTGTCTGGTATCTGATATCACTAGTATCT
LUSAD2.SEQ TGCCtGTTtGcAAGATCTGCATgTAGCAGTTTGTCTGGTATtTGcTACcAgTgGTATCT

LUCDNA.SEQ ..... 1980
LUSAD1.SEQ TTGTTTGATTCCCTAGCATCTCTGAAACCATCGGAC.AAGTAGGTGGTTTtagGACAAATT
LUSAD2.SEQ TTGTTTGATTCCCTAGCATCTCTGAAaCATCGGACCAAGTAtcTGGTT.AGGACAAATT

LUCDNA.SEQ ..... 2040
LUSAD1.SEQ TGGTTCATTGCGGCATTTTGTGTTGTATCGCGTATCATCTGGAAGAAGCAGACAGTTT
LUSAD2.SEQ TGGTTCATTGCGGCATTTTGTGTTGTATCGctGTATCgTCTGGAAGA.GCAGACAGTTT

LUCDNA.SEQ ..... 2100
LUSAD1.SEQ TGCAAAGTGGCATCAAGCTCAAGAAAGCAACGGCTAGAAGAAGTTCTACATCTGATGCTT
LUSAD2.SEQ TGCAAAGTGGCATCAAGCTCAAGAAAGCAACGGCTAGAAGAAGTTCTACATCTGATGcGT

LUCDNA.SEQ ..... AT 2160
LUSAD1.SEQ TCCTTTTGTTCCTTTGTGTGCTTTTGGACTTTGTTCCTTTTCCCTGTAGGATCCAAGAT
LUSAD2.SEQ TCCTTTTGTTCCTTTGTGTGCTTTTGGACTTTGTTCCTTTTCCCTGTAGGATCCAAGAT

LUCDNA.SEQ CCAAAAACAGAAAACAACCCCTACCTCGGTTTCATCTACACCTCATTCCAAGAGAGGGCA 2220
LUSAD1.SEQ CCAAAAACAGAAAACAACCCCTACCTCGGTTTCATCTACACCTCATTCCAAGAGAGGGCA
LUSAD2.SEQ CCAAAAACAGAAAACAACCCCTACCTCGGTTTCATCTACACCCcATTCCAAGAGAGGGCA

LUCDNA.SEQ ACGTTCATCTCCACGGAAACAGCCAGACTCGCCAAGGACCATGGGGACATGAAGCTG 2280
LUSAD1.SEQ ACGTTCATCTCCACGGAAACAGCCAGACTCGCCAAGGACCATGGGGACATGAAGCTG
LUSAD2.SEQ ACGTTCATCTCCACGGAAAcAGcGCCAGACTCGCCAAGGACCAcGGGGACATGAAGCTG

LUCDNA.SEQ GCGCAGATCTGCGGGATCATCGCAGCAGACGAGAAACGGCACGAAACCGCATAACCAAG 2340
LUSAD1.SEQ GCGCAGATCTGCGGGATCATCGCAGCAGACGAGAAACGGCACGAAACCGCATAACCAAG
LUSAD2.SEQ GCGCAGATCTGCGGGATCATCGCAGCAGACGAGAAgCGGCACGAAACaGCATAACCAAG

LUCDNA.SEQ ATCGTCGAGAAGCTCTTCGAGATCGACCCTGACGGTACAGTGCTGGCACTGGCGGACATG 2400
LUSAD1.SEQ ATCGTCGAGAAGCTCTTCGAGATCGACCCTGACGGTACAGTGCTGGCACTGGCGGACATG
LUSAD2.SEQ ATCGTCGAGAAGCTCTTCGAGATCGACCCTGACGGTACAGTgTGGctCTGGCGGACATG

LUCDNA.SEQ ATGAGGAAGAAGATATCGATGCCCCGCCACTTGATGTACGATGGAGAAGACGACAACCTC 2460
LUSAD1.SEQ ATGAGGAAGAAGATATCGATGCCCCGCCACTTGATGTACGATGGAGAAGACGACAACCTC
LUSAD2.SEQ ATGAGGAAGAAGATATCGATGCCCCGCCACTTGATGTACGATGGAGAAGACGACAACCTC

LUCDNA.SEQ TTCGACAATTACTCGTCAGTCGCTCAACGCATCGGGG...ATACTGCCAAGGATTATGCC 2520
LUSAD1.SEQ TTCGACAATTACTCGTCAGTCGCTCAACGCATCGGGGTGTATACTGCCAAGGATTATGCC
LUSAD2.SEQ TTCGACAATTACTCGTCgGTCGCTCAACGCATCGGGGTGTATACTGCCAAGGATTATGct

LUCDNA.SEQ GATATCCTGGAGTTCTTGGTGGGGAGGTGGAAAGTGGATGCTTTTACGGGGCTTTCCGGG 2580
LUSAD1.SEQ GATATCCTGGAGTTCTTGGTGGGGAGGTGGAAAGTGGATGCTTTTACGGGGCTTTCCGGG
LUSAD2.SEQ GATATCCTGGAGTTCTTGGTGGGGAGGTGGAAAGTGGATGCTTTTACGGGAcTTTCCGGG

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Figure 1 (cont.)

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LUCDNA.SEQ GAAGGGAACAAAGCTCAGGATTTTGTCTGCGGGCTTCCTGCGAGGATTCGAAAGTTGGAG 2640
LUSAD1.SEQ GAAGGGAACAAAGCTCAGGATTTTGTCTGCGGGCTTCCTGCGAGGATTCGAAAGTTGGAG
LUSAD2.SEQ GAAGGGAACAAAGCTCAGGAgTTTGTCTGtGGGCTTCCaGCGAGGATTCGAAAaTTGGAG

LUCDNA.SEQ GAGAGGGCTGCGGGGAGGGCAAAGCAAACGTCGAAATCTGTCCCGTTCAGCTGGATCTTC 2700
LUSAD1.SEQ GAGAGGGCTGCGGGGAGGGCAAAGCAAACGTCGAAATCTGTCCCGTTCAGCTGGATCTTC
LUSAD2.SEQ GAGAGGGCTGCGGGGAGGGCAAAGCAAACGTCGAAATCTGTCCCATTTCAGCTGGATCTTC

LUCDNA.SEQ AGCAGAGAATTGGTACTCTAAATGGAGTTTGCTTGAGAGT . AGAGTGTGGAATGATTGGAG 2760
LUSAD1.SEQ AGCAGAGAATTGGTACTCTAATGGAGTTTGCTTGAGAGTTAGAGTGTGGAATGATTGSAG
LUSAD2.SEQ AGCAGAGAATTGGTACTCTAATGGAGTTTGCCcGAGAGTT . GAGTGTGGAATGATTGGAG

LUCDNA.SEQ TATGATGGCAT 2771
LUSAD1.SEQ TATGATGGCAT
LUSAD2.SEQ TATGATGGCAT

Figure 1

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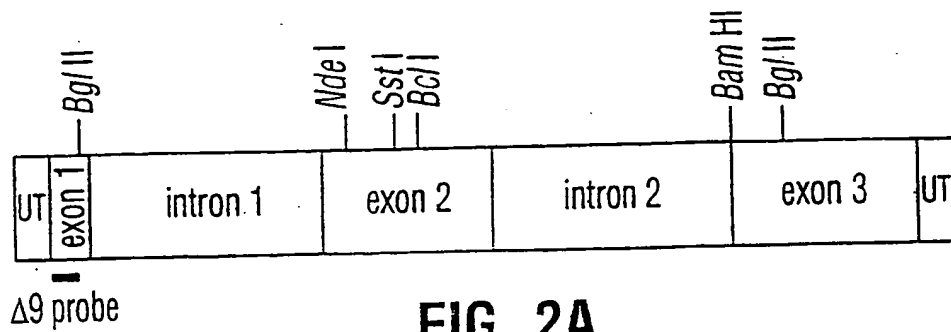


FIG. 2A

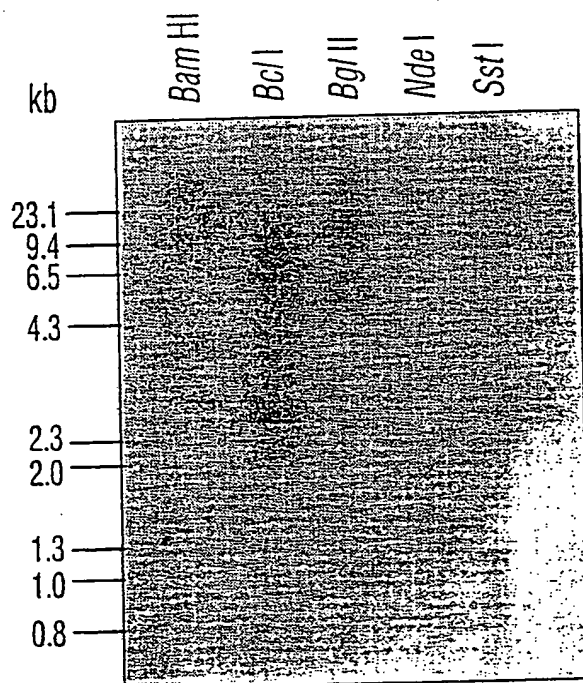


FIG. 2B

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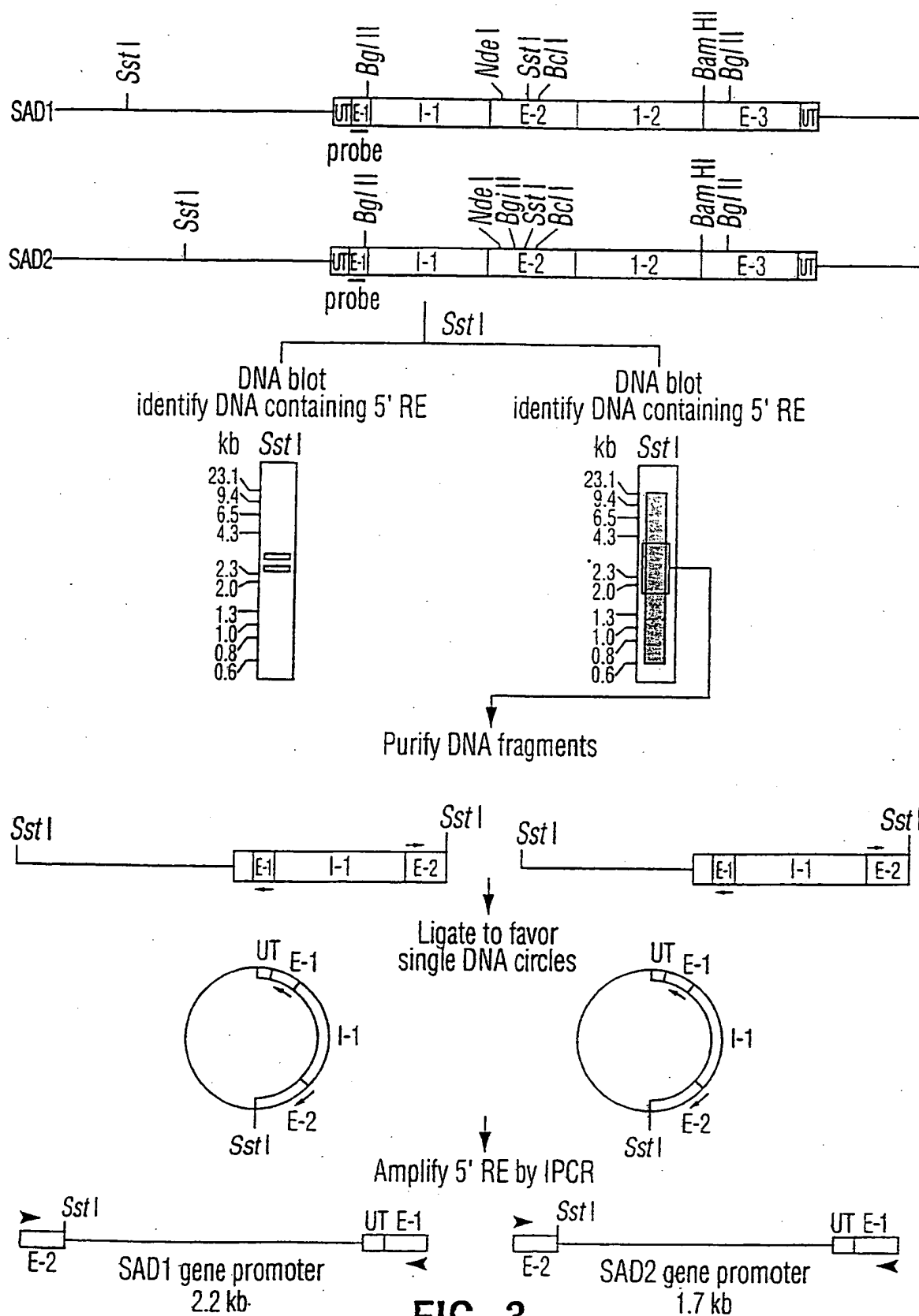


FIG. 3

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SstI

-1692 SAD1: GAGCTCTCAATGTAGTAACACAAAGCCTTCTGTCTTCTTTCTGTAACGTTCAATGCTAGA
 -1190 SAD2: -----C-T-T---CA-----G---T---

-1632 SAD1: AC.TTGTCTTCTTATAACTGTTTGTGCT....TCTTCAGCTAATGTTGGAGAAGGAT
 -1135 SAD2: --t-----T-----C---CA-gaagc-GA-----G-----

XhoI

-1578 SAD1: GGAGCCACGGAGATCCCGGTAAAGCAAAGGATGGATCGAGAGGAGACGGTGGCTCGAGAG
 -1075 SAD2: -----T-T-A-----A-----A-----

-1508 SAD1: AACATGGAAGCATTGCACAGAGCCGTCACGTTGGAAGTGCCTCATTGCGAGGCCCGTCT
 -1015 SAD2: T--G-----T-----C-T-C-----AG--TT-TT---

-1458 SAD1: CGGTATGGAACATTGGTGGTGGTGAAGAGAGG.AGAAAGATGCCGTAGTTCA
 -955 SAD2: -----A---C---C-----a-----TA-G---

-1399 SAD1: TCATCTACTGGGATGGATTGATCC.GCCAGCATGTTCTCCTCCCGAAATCGACCTGTCCC
 -895 SAD2: -----G---A-----a---G-----GG-C---

-1340 SAD1: TATTGATGACAATGTAACATCAATGTCAATCTCTGCAGATATCTGTTAGGATCAGGTCAT
 -835 SAD2: A-----T-T-----C-G-----

-1280 SAD1: GATTCTTTTTTGGTTGATTCTTGTGAATGTGAACATTGATGTAAGCTATTTGTTGTTGT
 -775 SAD2: -----

-1220 SAD1: AATA.....TCTGATTTTGTGTTGCTTTGATCAATCAAATAAATCTCGTTCAA
 -728 SAD2: ---tatgcattgat-G-----T-A-----G-----

NsiI

-1171 SAD1: CGCGATCATAAGCCTCTTTCATATTCATTTTGACGACTATGT....ATAGTCGTACAAAC
 -688 SAD2: -C-A-C---C--G-----A-atac--A-----

-1115 SAD1: TATTCGGTTAACTAATCTACATCAAGTCGGAATTAGCTAGACATTGTCAAGGAGGAGGAA
 -608 SAD2: -----GA-----G-----G-----

-1055 SAD1: AATATCAAGAAAATTGGATGAGGAAATCATAACCCCAATTCTGAAGCTGATTCTTCATCT
 -554 SAD2: -----G-----T---T-----

-995 SAD1: ATGATTTGAGTTTCGACTTTTGTGAGTCTCAACTGTGATTTGAGTTTCGACTTGATT
 -513 SAD2: -----T-----

-935 SAD1: TGGCTCTTTGATATTCCGAAATTA.....AATGCCTCCAAAGTGCTCTCTACTTGCG
 -489 SAD2: -----C-----aatgcctc-----

-893 SAD1: GTTGG.CCTGGTTTCANTGGCGAATCATTGAATGACAGAACTAGACAGCTACCAGGTGCAA
 -430 SAD2: -G--a-----A-----A-A-----

Fig. 4

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-824 SAD1: AAAACATTTGTT.....AATGTCTTCTTGCATTAAATGTCCATGTTTTCTGCATT.TT¹
-397 SAD2: -----G-----tacacgtcC---TA-----G-C-----g-A

BglIII

-773 SAD1: AATCTTTCCCAAACACCTAATATATAGCTTCATTGATCCTCTCTCCACGGTTGCAGAT
-339 SAD2: -----

-713 SAD1: CTCGTGCTGATAACACATACATGGCTACAAGACTCTAAAACGGTTCAAAGTGAAATTGT
SAD2: -----

-653 SAD1: TTTGGTGGTAGAGTTGTGTGTTTGGTGACTCGAAAGTTCTGGATTCCAATCCAGCATTCC
SAD2: -----

-593 SAD1: CCACAAAATAGACACCAACGTAGTGTATTATTACCGTCTTCTATCTTGTATTGACCGAGA
SAD2: -----

BamHI

-533 SAD1: GTTACGATATACTCCGACAAAAAAGACATCTTCCACATCATCAAATGGATCCGTAGTTA
SAD2: -----

-473 SAD1: GTGCAGTGGCTCGATTAAACATAAATGAAAAAGGAAAAAATTGCCTGAAATCGATGCTC
SAD2: -----

-413 SAD1: AAAACAAGTAGAAATTCATTCAAACATATTTAGACAAACACGATCATTTAGCATCATCAA
-324 SAD2: -----G-----G--GT---A--C---C-----

-353 SAD1: ATTAATAACAAGAGCAAACAATAAAGCACATAGCAAACATACAATAGTCGTCTTGCAAT
-287 SAD2: --C-----C-----T-----T-A-----

-293 SAD1: GTCATATGATAATAAGCCAGTGAAACCATGAAGCCCAAGTGAAGTGGTCAAGTGGGAGCT
-256 SAD2: -----T--T---G-----

HindIII

-233 SAD1: GAAAGCTT.....CCGAACCCAAGCCCCGCTACCGGGTTAGGACATACGACACGC
-220 SAD2: -----tcatcggtatT-----C-----

-182 SAD1: GACATGCTACGAAACTTAAAAATCGGTCACGCAGTTA.....ATGGAACAAATGAAACG
-188 SAD2: ..-C-----G---T---TA---cactcg--A---TG-----

-128 SAD1: CAACGACTATTAAGTGACCATTTTCAGAAATGAT.ATGAAAAAGTGACCATTTAGACAA
-130 SAD2: -----T-G-----A-----C---a---TG-C-----G--T--

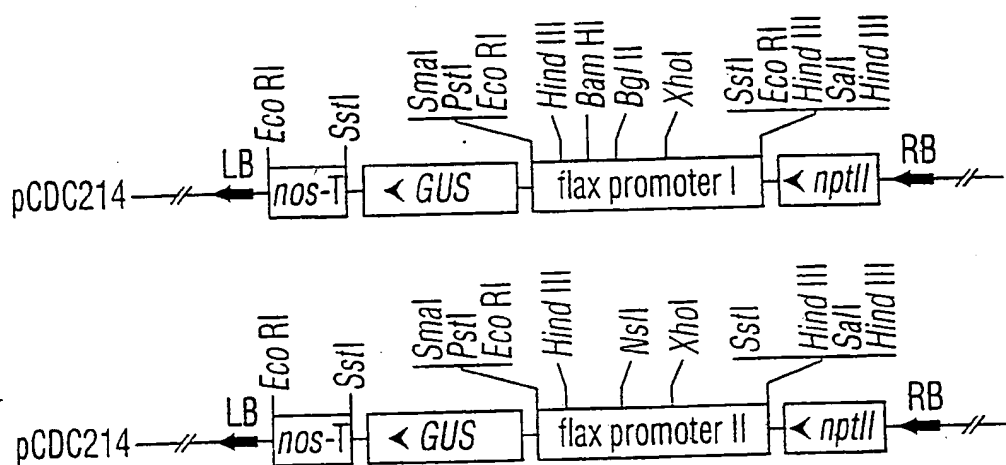
-69 SAD1 : ATGAGCAAAGAAAATAC.AGTGGCGAGTGCTGACATAATAAACCGAATGCAGGCGTTACC
-70 SAD2 : ----C--GG-----a-----G-----

+1

-10 SAD1 : ATCCAATTTTA
-10 SAD2 : -----

Fig. 4

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**FIG. 5**

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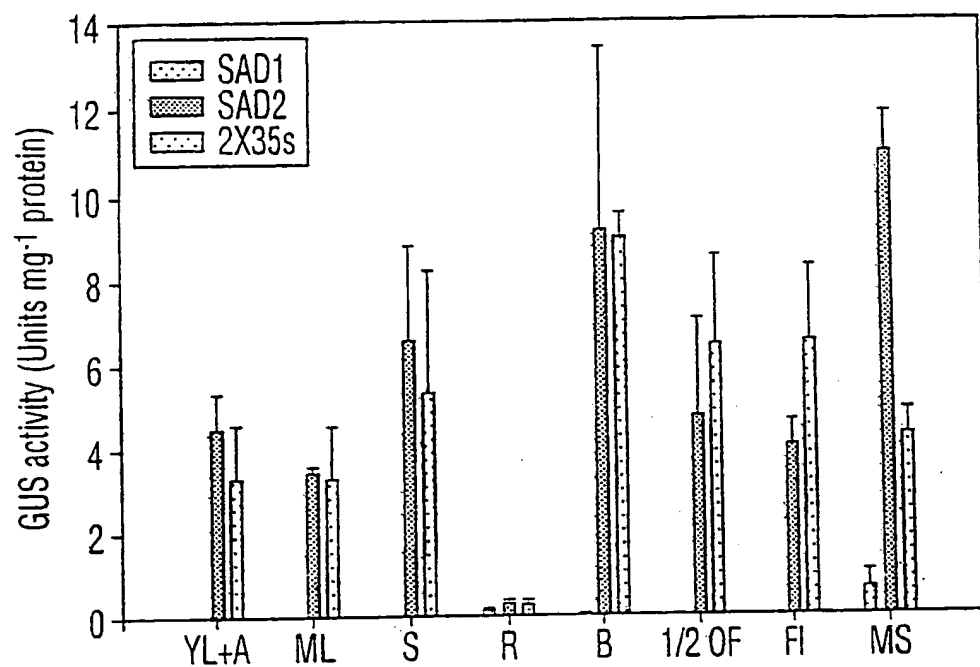


FIG. 6

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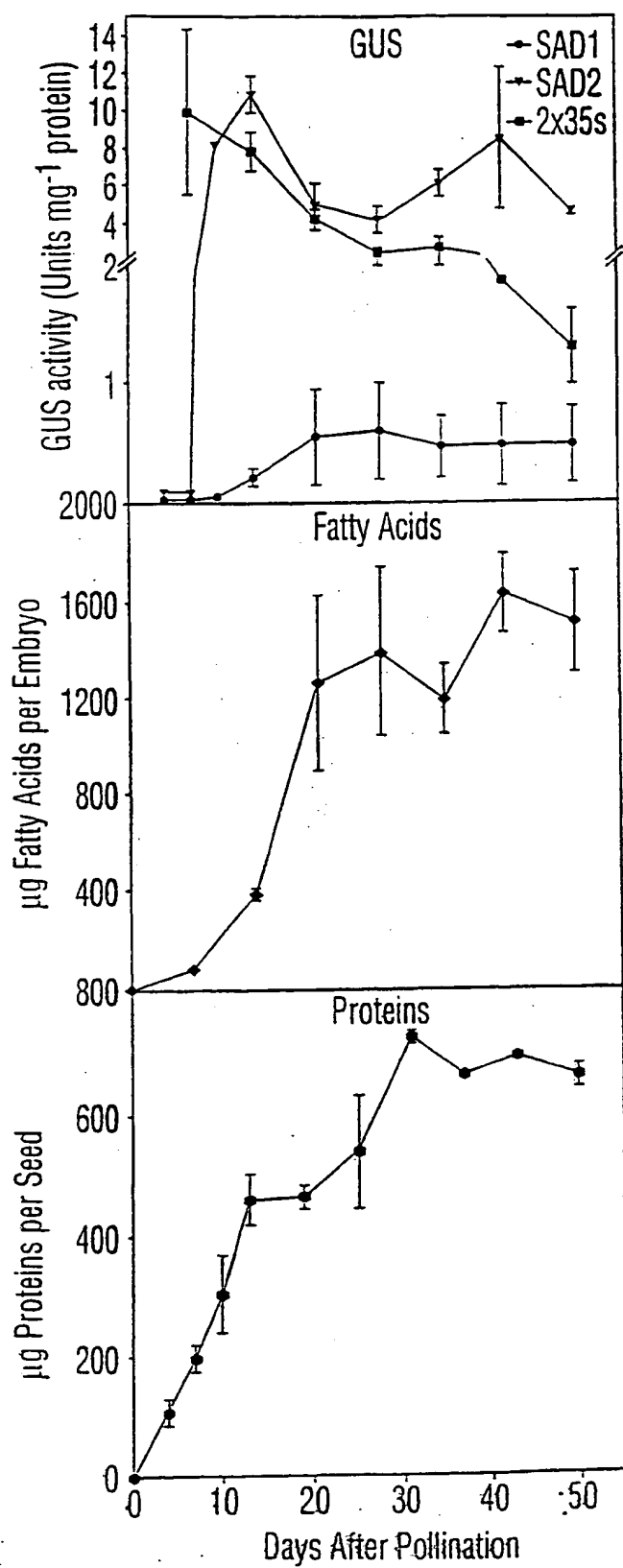


FIG. 7

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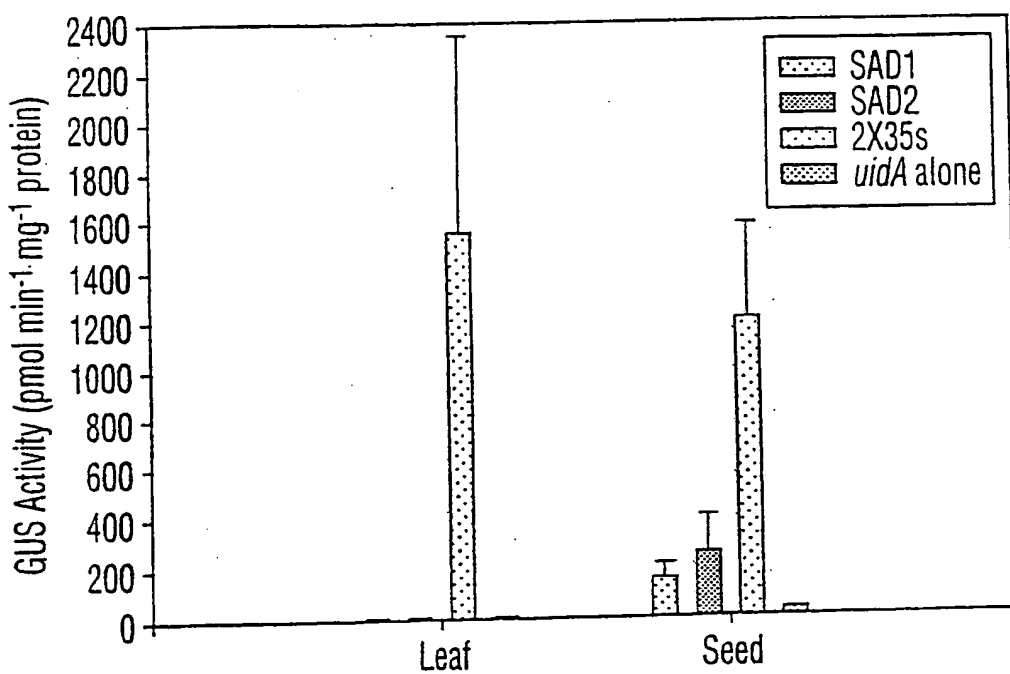


FIG. 8

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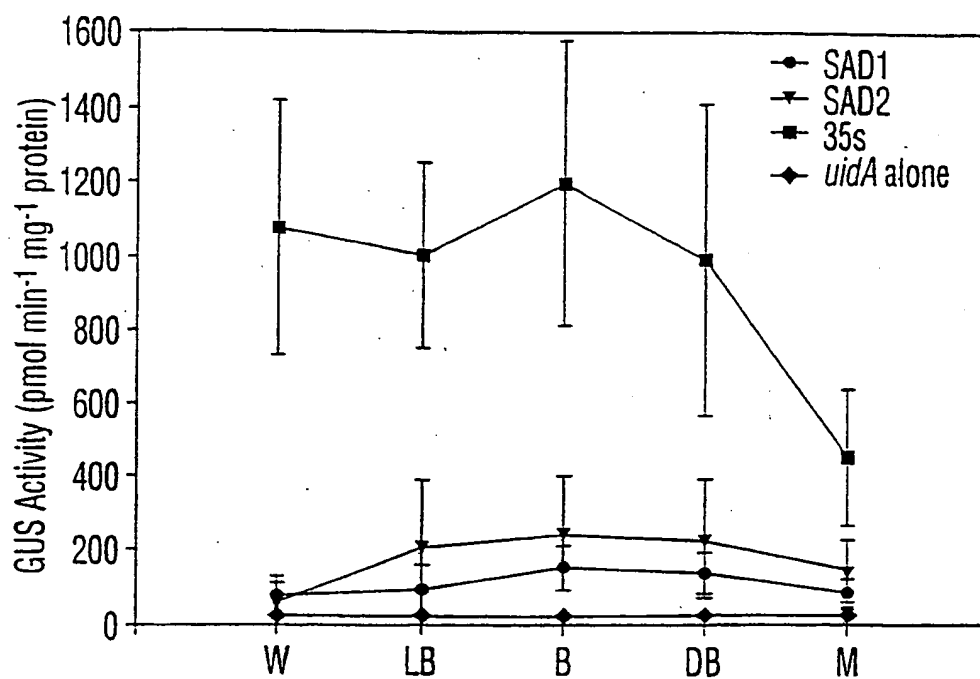
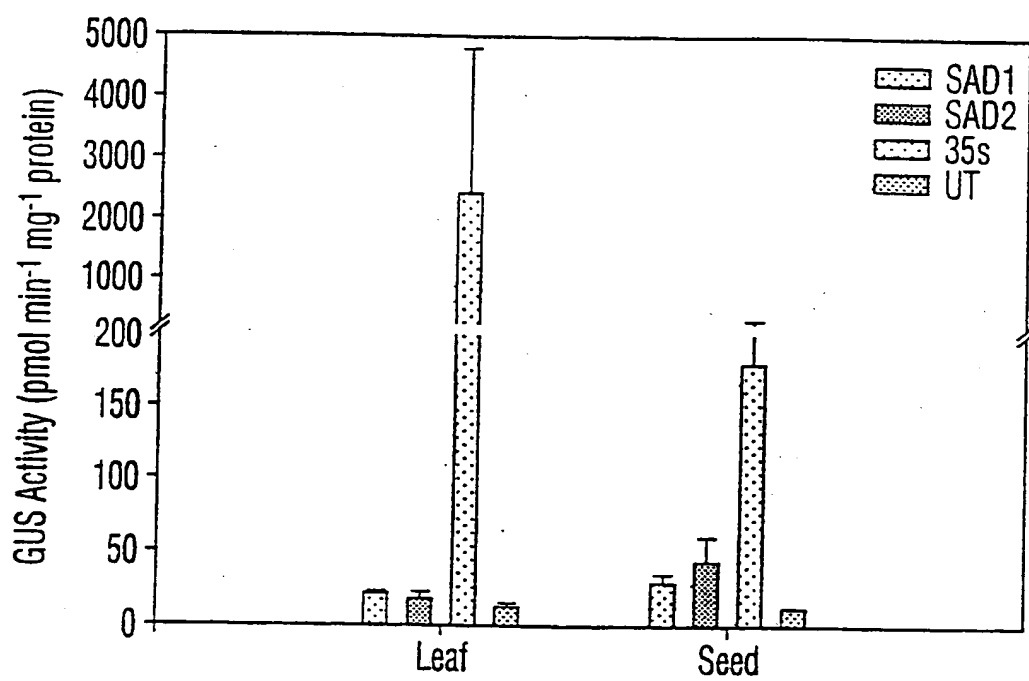


FIG. 9

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**FIG. 10**

INTERNATIONAL SEARCH REPORT

Internat'l Application No

PCT/CA 97/00812

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/82 C12N15/53 A01H5/00

According to International Patent Classification(IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N A01H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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Y	WO 91 13972 A (CALGENE INC) 19 September 1991 see the whole document	10,11,17

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☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

11 February 1998

Date of mailing of the international search report

03/03/1998

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INTERNATIONAL SEARCH REPORT

Inter nal Application No

PCT/CA 97/00812

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INTERNATIONAL SEARCH REPORT

International Application No

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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